JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2011. Vol. **5**(2), p. 761-768

The Influence of Metallo-Beta-Lactamase Production and Predisposing Risk Factors on Mortality in *Pseudomonas aeruginosa* Nosocomial Infections

K.V. Yogeesha Babu[1], H.P. Niranjan[1], V. Vijayanath[2], M.R. Anitha[3] and G.M. Raju[2]

[1]Department of Microbiology, S.S. Institute of Medical Sciences and Research Centre, Jnanashankara, NH-4 Bypass Road, Davangere - 577 005, India.
[2]Department of Forensic Medicine & Toxicology, [3]Department of Anatomy, VMKV Medical College, Salem, India.

(Received: 05 January 2011; accepted: 18 February 2011)

Metallo betalactamase (MBL) mediated resistance to carbapenems is an emerging threat in nosocomial infections caused by *Pseudomonas aeruginosa*. MBLs hydrolyze virtually all beta-lactam antibiotics leaving clinicians with no options for treatment, except Aztreonam and Polymyxin B. Very limited data available on impact of MBL producing *Pseudomonas aeruginosa* infections on mortality necessitated the present study. Of the 523 patients presenting with *P. aeruginosa*, 110 isolates from nosocomial infections (as per CDC definitions) were subjected to MBL detection by IMIPENEM+EDTA combined disc test. Incidence of Imipenem resistant Metallo betalactamase positive *P.aeruginosa*(IR-MBLP-PA) and Metallo-beta-lacatamse negative *P. aeruginosa*(MBLN-PA) infections was 21.82% and 9.38% respectively (P value 0.01 S) with six distinct antibiogram types circulating in the hospital. Mean duration of stay in the hospital before the isolation was 22±13.5 days. Overall mortality in *P. aeruginosa* infections was 13.63% (15/110). Increased mortality was observed in IR-MBLP-PA than in MBLN-PA (42.68% Vs 9.37% P value=0.01 S) with a mean duration of stay in ICU till death of 3.16±0.98 days indicating the severity of the infections. All deaths among IR-MBLP-PA infections were due to VAP as an underlying disease. Previous Imipenem therapy was significantly associated with IR-MBLP-PA infections (P value <0.001 HS) resulting in emergence and/or acquisition of IR-MBLP-PA. Other predisposing risk factors were significantly associated with IR-MBLP-PA infections. IR-MBLP-PA infections results in higher mortality than IR-MBLN-PA. VAP is the underlying disease in majority of deaths due to IR-MBLP-PA infections. Attributable mortality in IR-MBLP-PA infections, is partially mediated by production of Metallo-betalactamases, severity of underlying disease, predisposing risk factors, Multidrug resistance and Pan drug resistance, making IR-MBLP-PA isolate, a nosocomially successful and difficult to treat pathogen. Patients in whom Imipenem is selected as antipseudomonal antibiotic, the potential for emergence of IR-MBLP-PA strains should be anticipated, and in appropriate circumstance, routine culture and screening for MBL production should be performed to detect the emergence of IR-MBLP-PA strains. These findings can be generalized to other tertiary care hospitals with similar conditions. Further studies are needed to explore the population dynamics, virulence factors, an effective antibiotic and expression of MBL production *in vivo* of this serious pathogen.

Key words: Imipenem resistance, Metallo-beta-lactamases(MBLs), *Pseudomonas aeruginosa,* Predisposing risk factors.

Nosocomial infections caused by *Pseudomonas aeruginosa* are an unfortunate byproduct of advances in the modern medical treatment, invasive devices and increased survival of patients with decreased immune response. Acquired resistance in *P. aeruginosa* is far reaching and highly adaptable, can emerge rapidly

* To whom all correspondence should be addressed.
Email: dr.yogeshb77@yahoo.com
Mobile: +91-9916813119; Res: +91-8192241094

and progress through bacterial populations vertically and horizontally with relative ease[1]. Carbapenems have been used in clinical settings as last resort antibiotics for their broad spectrum antibacterial activity against various beta-lactamase producing Gram negative bacteria including extended spectrum beta-lactamases and Amp-C producers. [2] Resistance to Carbapenems is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and carbapenem hydrolyzing enzymes- Carbapenemases[3]. Acquired Metallo betalactamases (MBLs: IMP and VIM), a class B carbapenemases have recently emerged globally, since the first report from Japan in 1991. These are the most worrisome resistance mechanisms owing to their capacity to hydrolyze with the exception Aztreonam, all betalactam antibiotics, including carbapenems, the last resort antimicrobials for serious multidrug resistant gram negative infections[1]. Early detection of MBL producing *Pseudomonas aeruginosa* isolates is crucial to check the unnoticed spread within institutions. Situation is further complicated by nonavailability of standardized method proposed by CLSI for MBL detection[4]. Several nonmolecular screening tests viz., IMIPENEM+EDTA combined disc test, IMIPENEM-EDTA double disc synergy test, EDTA disc potentiation test and MBL-E test are being used for detection of MBL producing *P. aeruginosa*.[5] Most of the studies across the world highlight on incidence and prevalence of MBL positive *Pseudomonas aeruginosa* infections,[6,7] with very few studies highlighting its impact on mortality and morbidity[8,9,10].

With the increasing Imipenem resistant *Pseudomonas aeruginosa* infections at out hospital, it was decided to investigate whether MBL production was directly related to this resistance mechanism and epidemiology on these infections. Very little available data in the area of attributable mortality in Imipenem resistant MBL positive *P. aeruginosa* (IR-MBLP-PA) infections prompted us to conduct the present study.

MATERIAL AND METHODS

A prospective observational study of consecutive patients with *P. aeruginosa* nosocomial infections was performed at a tertiary care hospital for a period of one year. Isolates from nosocomial infections were included[11]. Polymicrobial infections and isolates from patients not fulfilling CDC criteria for nosocomial infections were excluded from the study[11].

Data were collected from medical records, computer database and in most of the cases in consultation with treating doctors. Different specimens from patients collected and processed according to standard laboratory procedures.[12] Susceptibility to Amikacin, Ciprofloxacin,, Gentamycin, Tobramycin, Piperacillin, Piperacillin- Tazobactam, Cefotaxime, Ceftazidime, Cefaperazone, Cefaperazone-Sulbactam, and Imipenem was determined by Kirby-Bauer's disc diffusion method according to CLSI guidelines[4]. Aztreonam, Polymyxin-B and Colistin were tested only against IR-MBLP-PA isolates.

P. aeruginosa isolates resistant to Imipenem were subjected to screening test for MBL production by IMIPENEM+EDTA combined disc test as described previously by Yong *et. al*.,[13]. Isolates with enhancement of zone size of more than or equal to 7mm between IMIPENEM+EDTA disc compared to IMIPENEM disc alone were considered as IR-MBLP-PA. MBL negative ATCC (27853) standard strain of *P. aeruginosa* was used as negative control, which did not show any zone of enhancement around IMIPENEM+EDTA combined disc.

Predisposing risk factors and length of hospital stay prior to isolation of IR-MBLP-PA isolate were analyzed statistically.

The outcome of IR-MBLP-PA infections was defined as CURE (Total resolution of signs and symptoms), improvement (Partial resolution) and deterioration followed by death.

Statistical analysis was done by Student "t" test by using SPSS windows version 13.0.

RESULTS

A total of 523 patients presented with isolation of *P. aeruginosa*, of them 283 community acquired infections, 60 contaminants or colonizers and 70 isolates from polymicrobial infections were excluded from the study. 110 isolates from nosocomial infections were included in the study. Relatively small sample size in this study was

inevitable since an attempt to increase the sample size by increasing the study period would have diluted the fast changing resistance scenario and epidemiology of IR-MBLP-PA infections.

Incidence of Imipenem resistant Metallo betalactamase positive *P. aeruginosa* isolates (IR-MBLP-PA) was 12.73% (14/110). Out of 24 Imipenem resistant isolates, 58.33% (14/24) were IR-MBLP-PA.

IR-MBLP-PA isolates were not observed in NICU

Overall in hospital mortality of patients with *P. aeruginosa* infections was 13.63% (15/110). Objective of the present study was to assess the influence of IR-MBLP-PA and MBLN-PA infections on mortality and morbidity. Relatively high crude mortality was observed among IR-MBLP-PA infections than MBLN-PA (42.86% versus 9.37%, P=0.01 S).

Among IR-MBLP-PA infections 6 patients presented with inadequate treatment followed by deterioration and death with VAP as underlying disease. Mean duration of stay in ICU till death was 3.167± 0.98 indicating severity of the disease. Eight patients who improved had mean duration of stay in ICU of 21 ± 4.95 indicating severe morbidity. Isolation of IR-MBLP-PA was from patients admitted for a long period of 22±13.5 days in hospital predisposing for emergence or acquisition of IR-MBLP-PA.

Antimicrobial therapy with Imipenem during previous 3 weeks (78.57% versus 7.29%, P< 0.001 HS) was more frequently associated with IR-MBLP-PA than MBLN-PA infections contributing to mortality, morbidity, acquisition of IR-MBLP-PA isolate and increased cost of the treatment. Predisposing risk factors were more commonly and significantly associated with IR-MBLP-PA than MBLN-PA infections.

Aztreonam and Polymyxin B were the drugs with least resistance, 14.29% and 0% respectively. Aztreonam was the most commonly used drug, due to higher cost and high frequency of adverse reactions seen with Polymyxin-B.

A total of six distinct antibiotic resistance profiles were observed in IR-MBLP-PA isolates. Profile 1 was commonest (Resistant to all drugs except Polymyxin B, Colistin and Aztreonam) causing 42.86% (6/14) infections.

DISCUSSION

Present study reported relatively high incidence of Imipenem resistance (21.82%) in nosocomial infections due to *P. aeruginosa*, with an increasing trend (14% in 2004, 15.6% in 2005, 17.2% in 2006 and 18% in 2007) [data not shown in tables]. This clearly indicates emergence and persistence of Imipenem resistant *Pseudomonas aeruginosa* isolates in the hospital. This tertiary care hospital mainly caters patients from surrounding rural areas, unlikely being treated with broad spectrum antibiotics like Imipenem acitng as a predisposing risk factor. Variable Imipenem resistance (8-63.74%) has been reported in different studies[2,3,5,6,7].

High incidence of IR-MBLP-PA infections (12.73%), constituting 58.33% of Imipenem

Table 1. Hospital area wise distribution of imipenem resistant mbl positive *P. aeruginosa* isolates

Hospital Area	Total no of *P. aeruginosa* isolates	Is-*P. aeruginosa* Isolates	Mortality in Is-*P. aeruginosa* (n=96)	Ir-*P. aeruginosa* isolates	No of IR-MBLP-PA Isolates	Mortality in IR-MBLP-PA Isolates
MICU	22	16	3	9	6	4
ICCU	16	12	1	5	4	2
Post operative ward	12	10	1	3	2	Nil
Burns ward	20	19	2	4	1	Nil
Neonatal icu	16	16	1	Nil	Nil	Nil
General ward	24	23	Nil	3	1	Nil
	110	96	9	24	14	6

NOTE;- IS-Imipenem sensitive, IR-MBLP-PA=Imipenem resistant MBL positive *Pseudomonas aeruginosa*, MBLN-PA= MBL negative *Pseudomonas aeruginosa*

Table 2. Clinical characteristics of fourteen patients with IR-MBLP-PA infections

S. No	Age(yrs) /sex	Diagnosis	Site of infection	Hospital stay prior to IR-MBLP-PA isolate	Duration of stay in ICU		Response to antibiotic therapy	Outcome of infection	death
					Till death	Till improve ment			
1	45/M	OP poisoning with respiratory failure	LRTI (VAP)	8	2	NA	Inadequate	Deterioration	+ ve
2	51/M	COPD with respiratory failure	LRTI (VAP)	16	3	NA	Inadequate	Deterioration	+ ve
3	22/F	OP poisoning with respiratory failure	LRTI (VAP)	7	4	NA	Inadequate	Deterioration	+ ve
4	49/M	Renal failure with trachea bronchitis	LRTI	26	NA	31	adequate	improvement	-ve
5	59/F	Intracerebral hemorrhage	LRTI (VAP)	11	4	NA	inadequate	Deterioration	+ ve
6	29/F	Tracheo bronchitis	LRTI	41	NA	17	adequate	improvement	-ve
7	52/M	MI with cardiac arrest	LRTI(VAP)	36	2	NA	Inadequate	Deterioration	+ ve
8	47/F	MI+LVF+ Respiratory failure	LRTI (VAP)	22	4	NA	Inadequate	Deterioration	+ ve
9	51/M	CCF with respiratory failure	Tracheo bronchits	17	NA	24	adequate	improvement	-ve
10	40/M	MI+cardiac arrest	Tracheo bronchitis	9	NA	24	adequate	improvement	-ve
11	51/F	Abdominal hysteretomy	Post operative wound infection	18	NA	14	adequate	improvement	-ve
12	22/M	Nephrectomy	Post operative wound infection	29	NA	18	adequate	improvement	-ve
13	31/M	Burns (40%)	Wound infection	15	NA	19	adequate	improvement	-ve
14	43/F	Intracerebral hemorrhage	Urinary tract infection	52	NA	21	adequate	improvement	-ve
				22±13.5(Mean±SD)		3.167±0.98days 21±4.95 days			

NOTE: F= Female , M=Male, NA=Not applicable , OP= Organophosphorus compound, LRTI- Lower respiratory tract infection , VAP= Ventilator associated pneumonia, UTI= Urinary tract infecti

Table 3. Distribution of predisposing risk factors in IR-MBLP-PA and IR-MBLN-PA infections

Risk factor	IR-MBLP-PA (n=14) (%)	MBLN-PA (n=96)(%)	P value *
Imipenem	11(78.57)	7(7.3)	<0.001 HS
Diabetes mellitus	8 (57.14%)	11(11.5)	0.001HNS
Long term iv cannulation	14 (100%)	22(22.9)	<0. 001 HS
Malignancy	2 (14.28%)	5 (5.2)	0.2 NS
Admission to icu of more than one week	10 (71.43%)	13(13.54)	<0.001 HS
Copd	7 (50%)	14(14.6)	0.01 S
Smoking	10 (71.43%)	33(34.4)	0.9 NS
Septicemia with multiorgan failure	6 (42.86%)	18 (18.8)	0.08 NS
Previous antibiotic treatment (In preceeding 3 weeks)	12(85.7%)	26 (26.1)	<0.001HS
Treatment with corticosteroids	8 (57.14%)	12 (12.5%)	0.001 HS
Anemia with hypoproteinemia	6 (42.86%)	48(50%)	0.6 NS
Urinary catheterisation	13 (92.86%)	19(19.8%)	<0.001HS
Congestive cardiac failure	3 (21.43%)	7(7.3%)	0.1 NS

*P value significant at d" 0.05

Table 4. Resistance rates of IR-MBLP-PA and IR-MBLN-PA isolates to different antibiotics

Antibiotic	IR-MBLP-PA(n=14)(%)	MBLN-PA(n=96)(%)
Gentamycin	14 (100)	78 (81.3)
Ciprofloxacin	10 (71.42)	60(57.6)
Piperacillin	10 (71.42)	65 (67.7)
Piperacillin + tazobactam	7 (50)	54 (51.84)
Cefotaxime	14 (100)	81(84.4)
Ceftazidime	10 (71.42)	57(59.3)
Cefaperazone	13 (92.86)	64 (66.7)
Cefaperzone + sulbactam	10 (71.42)	58(60.4)
Tobramycin	12 (85.71)	57(59.4)
Amikacin	10 (71.42)	57 (59.4)
Colistin	4 (28.57)	Not tested
Aztreonam	2 (14.29)	Not tested
Polymyxin b	0 (0)	Not tested

Table 5. A ntibiotic resistance profiles of 14 IR-MBLP-PA isolates

Profile	Antibiogram	Number (n)	Percentage(%)
1	R- All	6	42.86
2	R-G,PIP,Ce,Cs,Cs+Sul, ToS-C, PIP+Tz, Cz, Ak	2	14.28
3	R-C,G, Ce,Cz, Cs, ToS- PIP, PIP+Tz,Cs+Sul, Ak	2	14.28
4	R- G,C,Ce,Cs,Cs+Sul,To,AkS- PIP, PIP+Tz, Cz	2	14.28
5	R- G, PIP,PIP+Tz, Ce, Cz, AkS- C, Cs, Cs+Sul, To	1	7.14
6	R- G, PIP, Ce, Cz, Cs,AkS- C, PIP+Tz , Cs+Sul, To	1	7.14

NOTE: R- Resistant , S- Sensitive , G- Gentamycin, PIP= Piperacillin, PIP+Tz = Piperacillin+Tazobactam, Ce= Cefotaxime, Cs= Cefaperazone, Cs+Sul = Cefaperazone+Sulbactam, To= Tobramycin, Cz= Ceftazidime, Ak= Amikacin, Ce= Cefotaxime

resistant isolates was observed. However, this was an underestimation since polymicrobial infections were excluded from the study. IMIPENEM+EDTA combined disc test, a non-molecular screening test used in the present study is a sensitive and specific test clearly discriminating positive and negative results for MBL detection[5]. Though PCR is highly sensitive and specific test, is limited by high cost and nonavailability at all hospitals[5]. Significant proportions of Imipenem resistant *P. aeruginosa* isolates were found to be MBL producers by different Indian workers, Varaiya *et.al.,* 83.33% (50/60) and Hemalatha *et.al*, 87.5% (7/8)[7]. Most of the studies were limited by small sample size.

Distribution of IR-MBLP-PA infections was not uniform in our hospital. Five of the profiles 1, IR-MBLP-PA isolates were found in MICU and ICCU, contributing to majority of deaths due to IR-MBLP-PA infections. Most of the cases (71.42%) were from MICU and ICCU. An UTI (Profile 1) case with in situ catheter in general ward was a case shifted from ICU. Absence of IR-MBLP-PA infections from NICU was a direct result of strict infection control practices during last three years due to increasing mortality among infants with neonatal septicemia. Burden of IR-MBLP-PA infections was found to be just short of endemicity. The occurrence of an MBL-positive isolate in a localized hospital environment poses not only a therapeutic problem but also a serious concern for infection control management. The microbiology laboratory should promptly inform infection control team. The patient should be regarded as high risk, and appropriate isolation measures should be enforced. If necessary, patient's medical forms should indicate the high-risk nature of the infection, informing clinicians and other health care workers who may come in contact with the patient[2,3,14].

Significant finding was the higher mortality in IR-MBLP-PA infections (42.86%) compared with Imipenem sensitive *P. aeruginosa* infections 9.38% (P=0.01 S). Significantly higher mortality in MBL-PA patients than non-MBL patients (5.8% versus 1.2%) was reported by Hirakata et.al [9] and Laupland et.al (25% versus 13%). [10] Higher mortality rates and higher frequency of infections in such group indicates higher virulence of IR-MBLP-PA infections.[8,9,10] Although with higher virulence,57.14% of IR-MBLP-PA infections did not result in mortality and on the contrary 3 patients with 50% burns with MBLN-PA rapidly progressed to death. These findings yet again underscore the role of severe underlying disease contributing significantly to mortality and morbidity.

Ventilator associated Pneumonia (VAP) emerged as a single most important underlying disease leading to death. Though, attributable mortality due to VAP is questionable, 80% of mortality was observed in VAP due to IR-MBLP-PA than 10.42% in MBLN-PA. VAP due to IR-MBLP-PA significantly increases mortality since best diagnostic approach to therapy, rotational therapy and unconventional approaches to antimicrobial therapy remain uncertain[15]. VAP due to IR-MBLA-PA was an independent risk factor for mortality and morbidity in the current study.

In contrast to this, better prognosis with cure of two patients with IR-MBLP-PA nosocomial tacheobronchitis, two from post operative wound infections, and one each from burns and urinary tract infection signifies the role of less severe underlying disease as an important predictor of good prognosis.

Mortality in IR-MBLP-PA infections was associated with more frequent isolation of PAN DRUG resistant isolates (Profile 1) among IR-MBLP-PA compared to MBLN-PA (6 versus 2) and multidrug resistant isolates (14 versus 4). Paterson report that all of the IR-MBLP-PA isolates and 52% of MBLN-PA isolates were multidrug resistant, while 11% of IR-MBLP-PA isolates and 8% of MBLN-PA isolates were PAN DRUG resistant.[16] Clinicians were practically left with no option for treating patients with PAN DRUG resistant IR-MBLP-PA infections. This was due to antimicrobial resistance increasing the likelihood of an inadequate initial antibiotic regimen and of increased morbidity and mortality from inadequate initial treatment. As result, the mere possibility of infections due to antimicrobial-resistant pathogens necessitates broad spectrum initial empirical antimicrobial therapy, usually with combination of drugs including Imipenem. This increases the cost of treatment, the occurrence of adverse drug effects, and ironically, the local prevalence of antimicrobial resistance[15].

Approaching officially predicted "The end of antibiotics", it is certain that if physicians

do not decrease the overuse and misuse of antibiotics, the emerging IR-MBLP-PA infections with attended MDR and PDR isolates will worsen, while the era of "The end of Antipseudomonal antibiotics" will become a nosocomial nightmare[14].

In spite of adequate combinational antimicrobial therapy, 75% of VAP patients with IR-MBLP-PA isolates and 50% of patients with VAP due to MBLN-PA isolates expired. The specter of antimicrobial resistance complicated the management of nearly every patient with VAP. Aztreonam and Polymyxin–B were found to be most useful drugs. Zavascki et.al reported early institution of appropriate antimicrobial therapy as important predictor of good prognosis[8].

No extended survey with a series of human infections with MBL-positive isolates has been performed to determine the optimal treatment. Thus, suitable therapy for treating those infections remains unknown. Although metallo enzyme inhibitors like EDTA may be used in vitro, no MBL inhibitors are available for treating patients[14].

Previous antibiotic therapy with Imipenem was significantly associated with emergence and/or acquisition of IR-MBLP-PA isolates (78.57% versus 7.29%, P value<0.001 HS). Carmeli et.al. reports emergence of resistance to Imipenem in 8 patients, 7 of whom were treated with Imipenem.[16] Clinical emergence of resistant *P. aeruginosa* has been described during Imipenem therapy, ranging from 14-53% limiting future therapeutic choices and associated with increased mortality, morbidity and higher costs[15,16].

Predisposing risk factors (Table-3) associated with mortality, morbidity, emergence, and/or acquisition of IR-MBLP-PA isolates, were more commonly associated with IR-MBLP-PA than MBLN-PA infections. Prolonged stay in hospital prior to isolation of IR-MBLP-PA isolate was observed (Table-2).

Although, partially mediated by higher virulence, mortality in IR-MBLP-PA infections was associated with severe underlying disease, previous antibiotic therapy with Imipenem, MDR and PDR isolates in patients with multiple predisposing risk factors. Impact of IR-MBLP-PA isolate on mortality in an index patient could be assessed in the background of these factors prevailing in a patient at the time of isolation of IR-MBLP-PA.

Higher incidence of IR-MBLP-PA infections in the present study in a rural tertiary care hospital was probably due to rapid emergence and spread of IR-MBLP-PA isolates from critical care units of our hospital.

Majority of the IR-MBLP-PA infections in this study were caused by antibiotic resistance profile 1. Clonal relation and dissemination of IR-MBLP-PA strains could not be assessed as molecular typing was not done. In spite of small differences in susceptibility profiles, IR-MBLP-PA isolates usually have similar antibiotic resistance profiles regardless of the genotype of the enzyme[8]. Antibiogram typing of nosocomial isolates of IR-MBLP-PA will be useful in selecting appropriate antibiotic for empirical therapy. Successful clones will be widespread in nature and therefore predominate in the patient population, in whom variants accumulate drug resistance mechanism like Imipenem resistant metallo-beta-lactamases, that allow their transmission and persistence a hospital[17].

ICU is a "MELTING POT" for dissemination of MBL positive isolates since most of the isolates will be from ICU patients or related to ICU admission. It is likely that pseudomonads can transfer MBL genes through plasmids to Enterobacteriaceae, probably within a clinical environment. Rapid emergence and spread of MBL positive *P. aeruginosa* in hospital has been reported by several studies[2,3,10,14].

Timely identification of increased isolations of IR-MBLP-PA isolates achieved by active surveillance, implementation of isolation practices, timely reviewed hospital antibiotic policy appears to be crucial to limit the spread of IR-MBLP-PA isolates within a hospital. The spread of MBL genes is likely to escalate highlights the importance of international surveillance programs such as SENTRY, MYSTIC, Alexander Project and EARSS in reporting the emergence and epidemic spread of these remarkable but menacing enzymes.[14]

Conclusions of the present study are

1. Higher incidence of Imipenem resistant *P. aeruginosa* in a hospital necessitates the screening for detection of Metallo-beta-lactamase enzyme production.
2. Imipenem resistant MBL positive *P. aeruginosa* (IR-MBLP-PA) cause

significantly higher mortality compared to Imipenem sensitive *P. aeruginosa* infections.

3. Attributable mortality in IR-MBLP-PA infections, is partially mediated by production of Metallo-betalactamases, severity of underlying disease, predisposing risk factors, Multidrug resistance, Pan drug resistance, making IR-MBLP-PA isolate, a successful and difficult to treat pathogen. These findings can be generalized to other tertiary care hospitals with similar conditions
4. Patients in whom Imipenem is selected as antipseudomonal antibiotic, the potential for emergence of IR-MBLP-PA strains should be anticipated, and in appropriate circumstance, routine culture and screening for MBL production should be performed to detect the emergence of IR-MBLP-PA strains
5. Further studies are needed to explore the population dynamics, virulence factors, for an effective antibiotic and expression of MBL production *in vivo* of this serious pathogen

REFERENCES

1. Gerard D, Wright and Arlene D. Sutherland. New strategies for combating multidrug-resistant bacteria. *TRENDS in Molecular Medicine* 2007; **13**(6): 260-267.
2. Kurokawa H, Yagi T, Shibata N, Arakawa Y. Worldwide proliferation of Carbapenem resistant Gram negative bacteria. *Lancet.* 1999; **354**: 955.
3. Nordman P, Poirel L. Emerging Carbapenemases in gram negative aerobes. *Clin Microbial Infect.* 2002; **8**: 321-37.
4. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 7th Informational Supplement (M100-517), Wayne, PA: Clinical Laboratory Standards; 2007.
5. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of Metallo-Beta-lactamase producing *Pseudomonas aeruginosa*. *IJMM.* 2008; **26**(3): 233-237.
6. Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A. Emerging resistance to carbapenems in a tertiary care hospital in North India. *Indian J Med Res* 2006; **124**: 95-98.
7. Hemalatha V, Sekar U, Kamat V. Detection of Metallo-beta-lactamase producing *Pseudomonas aeruginosa* in hospitalized patients. *Indian J Med Res* 2005; **122**: 148-52.
8. Alexandre Prehn Zavascki, Afonso Luis Barth, Ana Lucia Saraiva Gonclaves, Ana Lucia Didonet More, Juliana Fernandez Fernandes, Anreza Francisco Martins et. al. The influence of Metallo-beta-lactamase production on mortality in nosocomial *Pseudomonas aeruginosa* infections. *Journal of Antimicrobial Chemotherapy* 2006; **58**: 387-392.
9. Hirakata Y, Yamaguchi T, Nakano M. Clinical and bacteriological characteristics of IMP-type Metallo-beta-lactamase producing *Pseudomonas aeruginosa*. *Clin Infect Dis* 2003; **37**: 26-32.
10. Laupland KB, Parkins MD, Church DL. Population-based epidemiological study of infections caused by carbapenem-resistant *Pseudomonas aeruginosa* in the Calgary health region: Importance of metallo-beta-lactamase (MBL) producing strains. *J Infect Dis* 2005; **192**: 1606-1612.
11. Garner JS, Jarvis WR, Emon TG. CDC definitions for nosocomial infections. *Am J Infect Control* 1988; 128-140.
12. Collee TG, Diguid JP, Fraser AG. Mackie and Mc Cartney practical Medical Microbiology. 14th ed. Edinburg: Churchill Livingstone; 2006.
13. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM Chang Y. Imipenem-EDTA disc method for differentiation of Metallo-beta-lactamase producing clinical isolates of Pseudomonas spp and Acinetobacter spp. *J Clin Microbiol* 2002; **40**: 3798-3801.
14. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta lacrtamases.the quiet before the storm?. Clin Microbiol Rev 2005;**18**:306-325.
15. David RP. Antimicrobial treatment of Ventilator associated pneumonia. *Respiratory care* 2005; **50**(7): 932-956.
16. Yehuda Carmeli, Nicolas Troillet, George M, Eliopoulos, Maththew H, Samore. Emergence of Antibiotic resistant *P. aeruginosa*: Comparison of risks associated with different antipseudomonal agents. *Antimicrobial agents & Chemotherapy* 1999: **43**(6): 1379-1382.
17. Altoparlak, U., S. Erol, M. N. Akcay, F. Celebi, and A. Kadanali. The time-related changes of antimicrobial resistance patterns and bacterial profiles of burn wounds and body flora of burned patients. *Burns;* 2004; **30**: 660-664.