

## Study of Imipenem Resistant Metallo-Beta-lactamase Positive *Pseudomonas aeruginosa* from Different Hospital Environmental Sources

K.V. Yogeesha Babu<sup>1</sup>, Amruta Kumara<sup>2</sup> and V. Vijayanath<sup>3</sup>

<sup>1</sup>Department of Microbiology, SS Institute of Medical Sciences and Research Centre, Jnanashankara, NH-4 Bypass Road, Davangere - 577005, India.

<sup>2</sup>Department of Microbiology, Government Medical College Mysore and Research Centre, Mysore, India.

<sup>3</sup>Department of Forensic Medicine, S.S.Institute of Medical Sciences & Research Centre Davangere - 577 005, India.

(Received: 10 December 2010; accepted: 20 January 2011)

Imipenem resistant Metallo -Beta-lactamase producing *Pseudomonas aeruginosa* (IR-MBLP-PA) are an emerging threat causing nosocomial infections with increased mortality and morbidity and with a potential to spread rapidly and cause outbreaks and epidemics. Very little data is available after review of literature on detection of IR-MBLP-PA from hospital environmental sources and their role as source and/or reservoir of nosocomial infections. The present study was conducted to detect IR-MBLP-PA from different hospital environmental sources from different areas of hospital, Antibio gram typing, to assess their role as source and /or reservoir of nosocomial infections and study the impact of infection control measures on environmental sources of IR-MBLP-PA. 460 environmental specimens collected and processed by standard laboratory procedures. Susceptibility testing done by Kirby-Bauer's disc diffusion method. IR-MBLP-PA detection was done by IMIPENEM+EDTA combined disc test. Antibio gram typing done. Association with clinical cases done by strain of same antibio gram type from environmental source and case. Impact of Infection control measures were assessed by percentage reduction of IR-MBLP-PA isolates from respective environmental sources.

Study reported an incidence of 24.78 %;5.65 % and 3.48%;1.08% for *Pseudomonas aeruginosa* and IR-MBLP-PA respectively, before and after strict infection control measures. High incidence of IR-MBLP-PA of 14.8% and 10.52% in suction apparatus and mops respectively, 11.53%, 8.89% and 8.24% in Burns ward, ICCU and MICU respectively was reported. Six of the eight IR-MBLP-PA antibio gram types from environmental sources could be associated with fourteen nosocomial infections with two strains with no association. Strain 1 (Resistant to all antibiotics used) was most common strain (30.76%) associated with six nosocomial infections during the study period. Sinks, suction apparatus were observed to be high risk sources and/or reservoirs of IR-MBLP-PA. MICU and ICCU were found to be high risk areas of environmental isolates necessitating periodic environmental sampling for their detection. Hospital air, aprons and gowns of health care workers, curtains, beddings and linen were not found to be important reservoirs of IR-MBLP-PA. Infection control measures according to CDC guidelines reduced the incidence of environmental IR-MBLP-PA isolates.

**Key words:** Imipenem resistant Metallo-Beta-lactamase positive *Pseudomonas aeruginosa* (IR-MBLP-PA), Environmental sources, Infection control measures.

---

\* To whom all correspondence should be addressed.

Mob.: +91-9916813119, Res.: +91-8192241094

E-mail: dr.yogeshb77@yahoo.com

Acquired resistance in *P. aeruginosa* is far reaching and highly adaptable, can emerge rapidly and can progress through bacterial populations vertically and horizontally with relative ease<sup>1,2</sup>. Acquired metallo-beta-lactamases (MBL: 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 of aztreonam, all betalactam antibiotics, including carbapenems, the last resort antimicrobial for serious multidrug resistant gram negative infection<sup>2,3</sup>. MBLs also represent a clinical threat due to their unrivalled spectrum of activity and their resistance to therapeutic serine beta-lactamase inhibitors and nosocomial infections associated with increased morbidity and mortality<sup>2,3</sup>.

The metabolic versatility of *P. aeruginosa* contributes to its broad ecological adaptability and ubiquitous distribution in the hospital environment and tendency to remain viable on both animate and inanimate objects around the patient, including antiseptic solutions<sup>3,4</sup>.

Rapid emergence and spread of MBL positive *P. aeruginosa* in hospital has been reported by several studies. The propensity of acquired MBL determinants to spread within the hospital, between different hospitals, into the community, and intercontinentally highlights the possibility that introduction of resistance genes in the nosocomial setting can be followed by a rapid dissemination among the different species of gram negative pathogens resulting in outbreaks of nosocomial infections<sup>1-4</sup>.

Little data is available regarding environmental reservoirs of IR-MBLP-PA isolates as a source of infection to patients. Early detection of MBL isolates is crucial to check the unnoticed spread within institutions<sup>2-4</sup>. Situation is further complicated by non availability of standardized method proposed by CLSI for MBL detection<sup>5</sup>. Several nonmolecular screening tests are used for detection of MBL producing *Pseudomonas aeruginosa*<sup>6</sup>.

Unlike other tertiary care hospitals, patients with IR-MBLP-PA infections shifted from other hospitals and colonized patients in our hospital were unlikely to be a source of IR-MBLP-PA isolates.

In view of the increasing IR-MBLP-PA nosocomial infection in this tertiary care hospital, the present study was undertaken to identify the environmental sources of IR-MBLP-PA with antibiogram typing and their association with nosocomial infections. Study was also conducted to assess the Impact of strict infection control measures on environmental sources of IR-MBLP-PA.

## MATERIAL AND METHODS

Prospective observational study of different environmental sources of Imipenem Resistant Metallo-Beta-lactamase positive *P. aeruginosa* (IR-MBLP-PA) was conducted for a period of one year in rural tertiary care hospital. A total of 460 random specimens were collected for targeted surveillance of different hospital environmental sources namely, Nebulizers (36), Tubings of ventilator (25), Antiseptic and disinfectant solution (48), Mops used for cleaning floors (38), Curtains, beddings and other linen (75), Aprons and Gowns of Health care workers (144), Faucets of sinks (40) and suction apparatus (54). Specimens from high risk areas of the hospital namely MICU, ICCU, NICU, BURNS WARD, OPERATION THEATRE and POST OPERATIVE WARD were collected according to standard procedures<sup>7</sup>. Specimens from ceilings, floor, walls, furniture and other environmental sources unlikely to come in contact with the patients were not included in the study. Specimens like stethoscope and other apparatus unlikely to come in contact with non-intact skin or mucus membrane, vasculature or tissues of the patients were also excluded from the study.

Forty seven Specimens were also collected from four general wards. Specimens from Aprons and Gowns were collected by RODAC PLATE METHOD. Rinse fluid was collected from Tubings of ventilator, Suction apparatus and Mops and Air microbiology investigated by settle plate method<sup>7</sup>.

All the swabs were inoculated into nutrient broth supplemented with 0.03% cetrimide (cetyltrimethyl ammonium bromide) and incubated at 37°C for 24 h. Subcultures were then performed on nutrient agar with 0.02% cetrimide, and the plates were incubated for 48 h at 37°C<sup>8</sup>. Colonies

producing a blue-green pigment were checked for oxidase reaction, and oxidase-positive isolates were subjected to identification by standard laboratory procedures<sup>9</sup>.

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.<sup>15</sup> 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.*<sup>10</sup> 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.

Typing of IR-MBLP-PA isolates was done by ANTIBIOGRAM TYPING. Association of IR-MBLP-PA environmental isolate with different nosocomial infections in different areas of the hospital was done by circumstantial evidence (Temporospacial association) and antibiogram type.

Repeat specimens were collected from the same hospital environmental sources, three months after strict infection control measures were implemented as per CDC guidelines.<sup>[11]</sup> Infection control measures were assessed in terms of percentage reduction in incidence of IR-MBLP-PA isolates from different hospital environmental sources.

## RESULTS

An Incidence of 24.78% (114/460) and 5.65% (26/460) was observed for *Pseudomonas aeruginosa* and IR-MBLP-PA respectively during one year study period in rural tertiary care hospital. Highest incidence of IR-MBLP-PA isolates was observed from suction apparatus (14.8%), followed by mops (10.52%) and sinks (10%). Neither *P. aeruginosa* nor IR-MBLP-PA was isolated from

**Table 1.** Distribution of environmental isolates of IR-MBLP-PA in different areas of the hospital

Environmental Source(n=number)	MICU	ICCU	NICU	BURNS ward	Operation theatre	POST ward	General ward	Total	% of PA/%IR-MBLP-PA]
Nebulizer (36)	2[1]/6 ¶	4[2]/6	2[0]/6	3[0]/2	2[0]/4	3[1]/6	1[0]/6	17[4]/36	47.22/11.11
Ventilator/Tubings (25)	4[1]/5	3[1]/5	4[0]/5	1[0]/2	0[0]/5	2[0]/3	Not tested	14[2]/25	56/8
Disinfectant/Antiseptics solutions (48)	2[0]/8	3[1]/8	2[0]/7	2[1]/5	2[0]/6	2[1]/4	1[0]/10	14[3]/48	29.17/6.25
Mops (38)	3[1]/6	2[1]/6	1[0]/4	1[1]/3	1[1]/10	2[0]/6	1[0]/3	11[4]/38	28.95/10.52
Curtains/Beddings/Linen(75)	0[0]/12	0[0]/15	0[0]/10	0[0]/10	0[0]/8	0[0]/10	0[0]/10	0[0]/75	0/0
Aprons and Gowns(144)	5[0]/30	8[0]/30	2[0]/20	2[1]/20	3[0]/26	2[0]/10	0[0]/8	22[1]/144	15.28/0.69
Sinks (40)	4[2]/8	2[1]/8	4[0]/6	2[1]/4	0[0]/6	3[0]/4	1[0]/4	16[4]/40	40/10
Suction Apparatus (54)	4[2]/10	3[2]/12	4[0]/6	3[2]/6	2[1]/8	3[1]/6	1[0]/6	20[8]/54	37.04/14.8
Total=460	24[7]/85	25[8]/90	19[0]/64	14[6]/52	10[2]/73	17[3]/49	5[0]/47	114[26]/460	24.78/5.65

NOTE: ¶ AIB/C ; A= No of *Pseudomonas aeruginosa*, B= No of IR-MBLP-PA isolates, C= No specimens collected from particular environmental source, MICU= Medical intensive care units, ICCU= Intensive Cardiac care unit, NICU= Neonatal intensive care unit  
PA= *Pseudomonas aeruginosa*, IR-MBLP-PA = Imipenem resistant MBL positive *P. aeruginosa*

**Table 2.** *P. aeruginosa* and IR-MBLP-PA isolates before and after infection control measures from different environmental sources of hospital

Environmental Source (n= number)	P. aeruginosa isolates		IR-MBLP-PA	
	Before IC Measures N= number (Percentage)	After IC Reduction Measures N= number (Percentage)	Percentage Reduction Before IC	Percentage Reduction
Nebulizer (36)	17(47.22)	4(11.11)	36.11	5.55
Ventilator Tubings (25)	14(56)	2(8)	48	8
Disinfectant/Antiseptics (48)	14(29.17)	0	29	6.25
Mops (38)	11(28.95)	1(2.63)	26.32	10.52
Curtains/Beddings/Linen(75)	0	0	0	0
Aprons and Gowns(144)	22(15.28)	2(1.39)	13.89	0.69
Sinks (40)	16(40)	3(7.5)	32.5	5
Suction Apparatus (54)	20(37.04)	4(7.41)	29.63	12.95

**Table 3.** *P. aeruginosa* and IR-MBLP-PA isolates before and after infection control measures from different environmental areas of hospital

Area of the Hospital (n)	No. of <i>P. aeruginosa</i>		Percentage Reduction	NO. OF IR-MBLP-PA		Percentage Reduction
	Before IC Measures	After IC Measures		Before IC Measures	After IC Measures	
MICU(85)	24(28.24)	4(4.71)	23.53	7(8.24)	3(3.53)	4.71
ICCU(90)	25(27.78)	3(3.33)	24.45	8(8.89)	2(2.22)	6.67
NICU(64)	19(29.69)	4(6.25)	23.44	0	0	0
BURNS WARD (52)	14(26.92)	1(1.92)	25	6(11.53)	0	11.53
Operation theatre(73)	10(13.39)	1(1.37)	12.02	2(2.74)	0	2.74
Post operative ward(49)	17(34.69)	3(6.12)	28.57	3(6.12)	0	6.12
General ward(47)	5(10.64)	0	10.64	0	0	0
Total (460)	114(24.78)	16(3.48)	21.3	26(5.65)	5(1.08)	4.57

NOTE; n= Number of environmental specimens collected, IR-MBLP-PA = Imipenem resistant Metallo Beta lactamase Positive *P. aeruginosa* MICU= Medical intensive care unit, ICCU= Intensive cardiac care unit , NICU= Neonatal intensive care unit , IC= Infection control

curtains, beddings and other linen. Lowest incidence of IR-MBLP- PA was from Aprons and gowns of healthcare workers (0.69% — 1/144).

Hospital area wise distribution of IR-MBLP-

PA was highest in burns ward (11.53%), followed by ICCU (8.89%) and MICU (8.24). Different environmental sources from NICU and General ward did not yield IR-MBLP-PA.

**Table 4.** Antimicrobial Resistance of 26 Hospital Environmental Isolates Of IR-MBLP-PA

Antibiotic	No resistant Isolates of IR-MBLP-PA	percentage of resistant IR-MBLP-PA
Imipenem	26	100
Gentamycin	26	100
Imipenem	26	100
Ciprofloxacin	19	73.08
Piperacillin	19	73.08
Piperacillin+tazobactam	19	73.08
Cefotaxime	26	100
Ceftazidime	19	73.08
Cefaperazone	25	96.15
Cefaperazone+sulbactam	25	96.15
Tobramycin	23	88.46
Amikacin	19	73.08
Colistin	8	30.77
Aztreonam	4	15.38
Polymyxin b	0	0

**Table 5.** Antibigram types of 26 IR-MBLP-PA isolates

Strain of IR-MBLP-pa	antibiogram	Number (n)	Percentage (%)	No of nosocomial infections caused by particular strain
1	R- Resistant to all	8	30.76	6
2	R- G, Pip,Pip+Tz, Ce, Cs, Cs+Sul, ToS- Cip, Cz, Ak	5	19.24	2
3	R- G, Cip, Ce, Cz,Cz+Sul, Cs, Cs+Sul, ToS- Pip, Pip+Tz,Ak	5	19.24	2
4	R- G, Cip, Ce, Cs, Cs+Sul, To, Ak S- Cz, Pip, Pip+Tz,	2	7.69	1
5	R- G, Pip, Pip+Tz, Ce, Cz, AkS- Cip, Cs, Cs+Sul, To	2	7.69	2
6	R- G, Pip, Ce, Cz, Cs, Ak, Pip+Tz, Cs+Sul	2	7.69	1
7	R- Pip, Pip+Tz, Cs, Cs+Sul, G, Ce, Cz, To, CipS- Ak	1	3.85	0
8	R –Ak, Pip, Pip+Tz, Cs, Cs+Sul, Ce, Cz, Cip, GS-To	1	3.85	0

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

Intervention in the form of hospital infection control measures according to CDC guidelines drastically decreased the incidence of *P. aeruginosa* from 24.78 % to 3.48%. and IR-MBLP-PA from 5.65% to 1.08%. IR-MBLP-PA from burns ward , operation theatre and post operative ward was reduced to undetectable levels .

Modest decrease in IR-MBLP-PA was noticed in nebulizer, sinks and suction apparatus at MICU. IR-MBLP-PA isolate persisted in MICU and ICCU in spite of good infection control measures . Although, with a highest incidence of IR-MBLP-PA isolates , no clinical cases due to this IR-MBLP-PA were observed from burns ward.

Statistical analysis could not be done even after Yates correction since most of the cell values were less than five .

Air microbiology by settle plate method from different areas of the hospital did not yield IR-MBLP-PA indicating hospital air as an unlikely source of *P.aeruginosa* or IR-MBLP-PA.

Highest resistance was observed with Gentamycin and Cefaperazone (100% and 96.15% respectively) . Incidence and Pan drug and multi drug resistant was found to be 8 and 12 respectively. Least resistance was noticed with Polymyxinn B. followed by Aztreonam and colistin.

Typing of environmental isolates of IR-MBLP-PA isolates by resistance profiles (ANTIBIOGRAM TYPING) revealed eight distinct strains. Strain 1 IR-MBLP-PA was most common (Resistant to all right) constituting 30.76% .

## DISCUSSION

Present study reported high incidence of *Pseudomonas aeruginosa* and Imipenem resistant Metallo-Beta-lactamase positive *P. aeruginosa* (IR-MBLP-PA) (24.78% Vs. 5.65%) from various environmental sources in different areas of the hospital. Twenty six IR-MBLP-PA environmental isolates belonging to 8 discrete strains by phenotypic characterization (ANTIBIOGRAM TYPING) were found to be source and/or reservoirs of nosocomial infections caused by IR-MBLP-PA. To our knowledge, this is the first description of a large-scale, hospital wide study of isolation of IR-MBLP-PA from different environmental sources in different areas of the hospital.

Awareness of entry of MBL producing *P. aeruginosa* isolates is the first step that clinical microbiologists can take to address this problem<sup>12</sup>. Distribution of environmental isolates was not uniform in the hospital. Most, but not all, of the environmental *P. aeruginosa* were from intensive care units and critical care areas of the hospital compared to General wards (80.77% Vs 19.23%) and all the IR-MBLP-PA isolates were confined to ICUs. Coexistence of IR-MBLP-PA isolates with non MBL producing *P. aeruginosa* was a worrisome finding as MBL resistance allele on a transferable conjugative plasmid could be readily mobilized to these isolates, further increasing the burden of IR-MBLP-PA environmental isolates in the hospital<sup>3</sup>.

Highest incidence of IR-MBLP-PA was in burns ward (11.53%) followed by ICCU (8.89%) and MICU (8.24%). None of the IR-MBLP-PA detected from NICU was the direct result of strict infection control measures being practiced due to high incidence of neonatal septicemia during last 4 years.

High incidence of IR-MBLP-PA was from suction apparatus (14.8%), Nebulizers (11.11%) and sinks (10%) from different areas of the hospital. Pitout *et.al.* reported MBL producing *P. aeruginosa* from 10 contaminated faucets (sinks) as reservoirs of outbreak in ICU and acute care centre. <sup>[12]</sup> Crespo *et. al* have reported an incidence of 20% for *P. aeruginosa* and IR-MBLP-PA from small study group of 60 environmental sources, 33 sinks, 5 stethoscopes and 22 tubing devices<sup>8</sup>. 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<sup>3</sup>.

Twelve IR-MBLP-PA isolates exhibited cross resistance to most of the antipseudomonal antibiotics (MDR) and 8 were PAN DRUG resistant. Aztreonam, Colistin and Polymyxin B were retained good sensitivity. Paterson reported that all of the IR-MBLP-PA and 52% of IR-MBLN-PA were multidrug resistant, while 11% of IR-MBLP-PA and 8% of IR-MBLN-PA were PAN DRUG resistant<sup>13</sup>.

Six of the eight strains of environmental IR-MBLP-PA isolates were associated with 14 nosocomial infections during study period from different ICUs. This association could be established by circumstantial evidence,



temporospatial association and identical strains from environmental sources and clinical cases by antibiogram typing. Though the routes of transmission of the IR-MBLP-PA remained unclear, IR-MBLP-PA recovered from these areas of hospital environment were found to be the possible source of increasing nosocomial infections due to existence of ample of opportunities in ICU s for transmission.

Strain 1, IR-MBLP-PA isolate (PAN DRUG RESISTANT) present in nebulizer, tubings of ventilator and sink in the MICU was associated with four clinical cases of ventilator associated pneumonia resulting in death and two cases of nosocomial tracheobronchitis with severe morbidity. The unique problem with MBLs is their unrivalled broad-spectrum resistance profile. Clinicians are practically left with no option for treating patients with PAN DRUG resistant IR-MBLP-PA infections. Selection of antibiotic for empirical therapy for IR-MBLP-PA should be based on antibiogram of locally prevalent IR-MBLP-PA environmental strains. Antimicrobial resistance increases 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<sup>14</sup>.

Strain 2 of IR-MBLP-PA isolate from tubings of ventilator, strain 3 and strain 4 were isolated from environmental source of OPERATION THEATRE and POST OPERATIVE WARDS. These strains could not be linked to nosocomial infections caused by IR-MBLP-PA isolates in these areas of the hospital. This underlines the importance of strict infection control measures preventing the transmission of IR-MBLP-PA isolate from environmental source to susceptible patients. It is not possible to state that strains isolated only from the hospital environment during relatively short period of the investigation have never caused infection or would never do so if given the opportunity. This does not rule out the possibility of transmission of these strains to susceptible

patients in future if strict infection control measures are not practiced.

Few strains of IR-MBLP-PA from nosocomial infections were not found in any of the environmental sources of the hospital. This necessitates further workup to identify any other environmental source of IR-MBLP-PA isolates in the hospital. Though there is little evidence to suggest healthy carriers among health care workers as source of IR-MBLP-PA isolates, the possibility cannot be ruled out. Crespo et.al reported a patient shifted from another hospital as a source of clinical infections<sup>8</sup>. Tsakris *et al.* reported community acquired IR-MBLP-PA isolates from feces from healthy adults in community resulting in the community acquired IR-MBLP-PA infections. Some as yet unknown environmental species also could be the sources of the mobile metallo-beta-lactamase determinants that recently appeared among gram negative pathogens<sup>15</sup>.

Reduction in incidence of *P. aeruginosa* and IR-MBLP-PA, 21.3% and 4.57% respectively was achieved following strict infections control measures for three months. Significant reduction in incidence was observed in highrisk areas, MICU, ICCU, POST OPERATIVE WARD and BURNS WARD. Maximum percentage of reduction of *P. aeruginosa* and IR-MBLP-PA was observed in suction apparatus and mops. (Table 3). Similar observations were made by Pitout *et.al* at Calgary health region<sup>12</sup>.

Neither environmental IR-MBLP-PA isolates nor clinical infections due to IR-MBLP-PA isolates were observed in NICU. However, a breach in the strict infection control measures being practiced may introduce IR-MBLP-PA isolates into NICU from other ICUs as IR-MBLP-PA rapidly spread in the hospital after their entry<sup>3,4,8,12</sup>.

The ICU was clearly the "MELTING POT" for emergence, persistence and spread of IR-MBLP-PA isolates. At Calgary health region. Pitout et. al. have demonstrated ease with which MBL producing strains accompanied patients when transferred to other acute care centers of the same hospital, nursing homes or the community. However, these strains did not cause an outbreak outside acute care center, underlining yet again the importance of environmental reservoirs as a source of nosocomial outbreaks due to MBL producing *P. aeruginosa*<sup>12</sup>.

MBL-producing *P. aeruginosa* bacteria are slowly but steadily increasing within hospitals, causing outbreaks and/or hyper endemic situations in several centers, mostly in the Far East and South of Europe<sup>15</sup>. Japanese reports denote the wide dispersion of MBL genes principally carried on class 3 integrons in 16 different species of gram negative bacteria, which has also been indicated in a more local setting<sup>3</sup>.

Timely detection of IR-MBLP-PA isolates from different environmental sources, achieved by active surveillance and hospital infection control measures, appears to be crucial in decreasing the incidence of IR-MBLP-PA nosocomial infections and the spread of these strains in the hospital.

### CONCLUSIONS

1. This study underscores, the role of hospital environment as potential source and/reservoir of IR-MBLP-PA isolates necessitating periodic environmental sampling in high risk areas of the hospital for detection of Imipenem Resistant Metallo-Beta-lactamase positive *Pseudomonas aeruginosa* (IR-MBLP-PA)

2. Suction apparatus and sinks were found to be most important sources of IR-MBLP-PA isolates with a high incidence in burns ward, MICU and ICCU necessitating strict infection control measures to prevent their spread to other areas of the hospital

3. Curtains, beddings, linen, Aprons and Gowns of health care workers and hospital air were not found to be important sources of IR-MBLP-PA isolates

4. Antibiotic resistance pattern of environmental IR-MBLP-PA isolates (ANTIBIOGRAM TYPING) help in choosing initial antibiotic for treatment

5. Strict infection control measures brought down the incidence of environmental *P. aeruginosa* and IR-MBLP-PA associated with decrease in nosocomial infections due to this isolate

### REFERENCES

- Gerard D, Wright and Arlene D. Sutherland. New strategies for combating multidrug-resistant bacteria. *TRENDS in Molecular Medicine* 2007; **13**(6):260-267.
- Kurokawa H, Yagi T, Shibata N, Arakawa Y. Worldwide proliferation of Carbapenem resistant Gram negative bacteria. *Lancet* 1999; **354**: 955.
- Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta lactamases. the quiet before the storm?. *Clin Microbiol Rev* 2005; **18**: 306-325
- 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.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 7<sup>th</sup> Informational Supplement (M100-517), Wayne, PA: Clinical Laboratory Standards; 2007.
- 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.
- Aaaaaa Sehulster LM, Chinn RYW, Arduino MJ, Carpenter J, Donlan R, Ashford D, Besser R, Fields B, McNeil MM, Whitney C, Wong S, Juranek D, Cleveland J. Guidelines for environmental infection control in health-care facilities. Recommendations from CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). Chicago IL; American Society for Healthcare Engineering/American Hospital Association; 2004.
- M. P. Crespo N., Woodford A., Sinclair, M. E., Kaufmann, J Turton J., Glover J. D. *et al.* Outbreak of Carbapenem-Resistant *Pseudomonas aeruginosa* Producing VIM-8, a Novel Metallo- $\beta$ -Lactamase, in a Tertiary Care Center in Cali, Colombia. *J. Clin. Microbiol.* 2004; **42**(11): 5094-5101
- Collee TG, Diguid JP, Fraser *Pseudomonas aeruginosa* AG Mackie and Mc Cartney practical Medical Microbiology. 14<sup>th</sup> ed. Edinburg: Churchill Livingstone; 2006.
- 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.
- William A. Rutala, David J. Weber, and the Healthcare Infection Control Practices Advisory Committee (HICPAC). Guideline for



- Disinfection and Sterilization in Healthcare Facilities; 2008.
12. Johann D. D., Pitout, Barbara L C., Daniel B., Gregson, Kevin B. et al. Molecular epidemiology of Metallo- $\beta$ -lactamase (MBL)-producing *Pseudomonas aeruginosa* in the Calgary Health Region: the emergence of VIM-2 producing isolates. *J. Clin. Microbiol.* 2010; **45**(2): 294-298.
  13. Yehuda Carmeli, Nicolas Troillet, George M, Eliopoulos, Maththew H, Samore. Emergence of Antibiotic –resistant *Pseudomonas aeruginosa*: Comparison of risks associated with different antipseudomonal agents. *Antimicrobial agents and Chemotherapy* 1999; **43**(6):1379-1382.
  14. David RP. Antimicrobial treatment of Ventilator associated pneumonia. *Respiratory care* 2005; **50**(7): 932-956.
  15. Athanassios Tsakris, Aggeliki Poulou, Ioulia Kristo, Theodore Pittaras, Nicholas Spanakis, Spyros Pournaras et al. Large Dissemination of VIM-2–Metallo- $\beta$ -Lactamase–Producing *Pseudomonas aeruginosa* Strains Causing Health Care-Associated Community-Onset Infections. *J. clin. Microbiol.* 2009; **47**(11): 3524-3529.