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Study of Imipenem Resistant *Pseudomonas aeruginosa* and Associated Predisposing Risk Factors in a Rural Tertiary Care Hospital

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Imipenem resistance is an emerging threat in nosocomial infections caused by *Pseudomonas aeruginosa*. Imipenem resistant *Pseudomonas aeruginosa* (IR-PA) with a increased mortality and morbidity worsen the situation by virtue of their multi-drug resistance and thus limit therapeutic options. Very limited data available on IR-PA nosocomial infections and associated predisposing risk factors necessitated the present study. Of the 523 patients presenting with *P. aeruginosa*, 110 isolates from nosocomial infections (as per CDC definitions) were analyzed by Kirby-Bauer's disc diffusion method of antimicrobial susceptibility testing for the detection of IR-PA. Predisposing risk factors were analyzed by student "t" test, and "z" test for proportions, using SPSS for windows, version 13.0. Incidence of Imipenem resistant *Pseudomonas aeruginosa* infections was 21.82% with eight distinct antibiogram types circulating in the hospital. Overall mortality in *P. aeruginosa* infections was 13.63% (15/110). Increased mortality was observed in IR-PA than in IS-PA (33.3% Vs 8.14% 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. Majority of deaths among IR-PA infections were due to VAP as an underlying disease. Previous Imipenem therapy was significantly associated with IR-PA infections (P value <0.001 HS) resulting in emergence and/or acquisition of IR-PA. Other predisposing risk factors were significantly associated with IR-PA infections. IR-PA infections results in significantly higher mortality than IS-PA. VAP is the underlying disease in majority of deaths due to IR-PA infections. Attributable mortality in IR-PA infections, is partially mediated by Imipenem resistance, severity of underlying disease, predisposing risk factors, Multidrug resistance and Pan drug resistance, making IR-PA isolate, a successful and difficult to treat pathogen. Patients in whom Imipenem is selected as antipseudomonal antibiotic, the potential for emergence of IR-PA strains should be anticipated, and in appropriate circumstance, routine culture and sensitivity should be performed to detect the emergence of IR-PA strains. These findings can be generalized to other tertiary care hospitals with similar conditions.

Key words : Imipenem resistant *Pseudomonas aeruginosa* (IR-PA), Imipenem sensitive *Pseudomonas aeruginosa* (IS-PA), 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

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reaching and highly adaptable, can emerge rapidly and progress through bacterial populations vertically and horizontally with relative ease[1]. *P. aeruginosa* is a successful nosocomial pathogen due to administration of broad spectrum antibiotics, instrumentation, and intrinsic resistance of the organism to multiple antibiotics[2]. The introduction of Carbapenems into clinical practice was of great help in the treatment of Penicillin resistant and Cephalosporin resistant gram negative infections since Carbapenems are resistant to hydrolysis by most Beta-lactamases[1]. Imipenem resistant *P. aeruginosa* isolates in ICU settings, cause increased morbidity and mortality in patients with underlying disease, or limit therapeutic options due to high degree of multi-drug resistance[3]. *P. aeruginosa*, normally a saprophyte, cause serious infections in immunocompromised and hospitalized patients especially those admitted to ICUs[4]. Kirby-Bauer disc diffusion test is a simple, cheap and highly reproducible yet a sufficiently sensitive and specific test for detection of Imipenem resistance .

Paucity of information on Imipenem resistant *P. aeruginosa* nosocomial infections necessitated the present study. Present study was undertaken to determine the incidence of Imipenem resistant *P. aeruginosa* and role of predisposing risk factors in patients admitted to a rural tertiary care hospital.

MATERIAL AND METHODS

A prospective observational study of consecutive patients with *P. aeruginosa* nosocomial infections was performed at a rural tertiary care hospital for a period of one year. Isolates from nosocomial infections were included[5]. Polymicrobial infections and isolates from patients not fulfilling CDC criteria for nosocomial infections were excluded from the study[5].

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[6]. 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[7]. *P. aeruginosa* were considered as Imipenem resistant when the zone of inhibition around Imipenem disc was d" 13 mm. Aztreonam, Polymyxin-B and Colistin were tested only against IR-PA isolates.

Severity of patient's condition was assessed by mean duration of stay in ICU till cure and till death, number of episodes of complications related to infections. Predisposing risk factors of IR-PA isolate were analyzed statistically.

Statistical analysis was done by "z" test for proportions and 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 Imipenem resistant P.aeruginosa infections.

One hundred and forty eight specimens collected from 110 patients from different areas of the hospital yielded *P. aeruginosa*. A total of 31 Imipenem resistant *P. aeruginosa* were isolated from different clinical specimens, often with multiple specimens yielding IR-PA from a single patient. Overall, 24 isolates from different specimens yielded IR-PA.

Overall incidence of IR-PA was 21.82% (24/110) with highest incidence in MICU 6.08% (9/148). IR-PA were not isolated from NICU. Specimen wise distribution of IR-PA was highest in lower respiratory tract secretions 7.43% (11/148) followed by blood 6.75% (10/148). Incidence of IR-PA from general wards was 4.05%(6/148)

In the present study 66.67% (8/12) of patients with IR-PA infections suffering from ventilator associated pneumonia (VAP), rapidly progressed to death with mean duration of stay in ICU till death of 3.167 +/- 0.98 days indicating the severity of infection. 33.3% (4/12) improved

Table 1. Distribution of imipenem resistant *Pseudomonas aeruginosa* in areas of the hospital from various clinical specimens

Area of the Hospital (N= number of Patients)	Blood	Urine	Sputum and others	Exudates	Total
Micu(22)	12[2]	2[1]	12[6]	2	28[9]
Iccu(16)	6[1]	2	10[4]	nil	18[5]
Post operative ward (12)	4[2]	4	nil	12[3]	20[5]
Burns ward(20)	8[2]	2	8	14[4]	32[6]
Nicu(16)	10	4	4	nil	18[0]
General ward(24)	8[3]	14[2]	6[1]	4	32[6]
Total	48[10]	28[3]	40[11]	32[7]	148[31]

Note; micu= medical intensive care unit, iccu=intensive cardiac care unit, nicu= neonatal intensive care unit,

Table 2. Distribution of prognostic factors in IR-PA and IS-MBLN-PA patients

Prognostic factor	IR-PA (n=24) (percentage)	IS-PA (n=86) (percentage)	P value *
Mortality	8 (33.3%)	7(8.14%)	0.01 S
Mean duration of stay till death	3.167+/-0.98	16+/-2.82	**P** <0.001HS t=10.74
Mean duration of stay till improvement	21+/-4.95	6.125 +/2.1	P <0.001HS t=7.87
No. of episodes of complications related to infection	5.85+/-1.65	3.7+/-1.31	P < 0.001HS t=4.6
Previous antibiotic treatment with imipenem	14(58.33%)	4(4.65%)	P 0.001 S

NOTE: IR-PA = Imipenem resistant *Psedudomonas aeruginosa*
IS-PA = Imipenem sensitive *Pseudomonas aeruginosa*

Table 3. Association of predisposing risk factors in imipenem senstive and imipenem resistant *Pseudomonas aeruginosa* nosocomial infections

Predisposing Risk Factor	Imipenem-resistant *P. aeruginosa* Infections (24)	Imipenem Sensitive *P. aeruginsoa* Infections (86)	P Value
Previous antibiotic therapy (excluding Imipenem)	22	16	<0.001 HS
Diabetes mellitus	11	8	<0.001 HS
Long term iv cannulation	23	13	<0.001 HS
Malignancy	2	5	0.64 NS
Admission to ICU of more than 1 week	16	7	<0.001 HS
Copd	10	11	0.007 S
Smoking	18	25	<0.001 HS
Septicemia with multiorgan failure	8	16	0.16 NS
Treatment with steroids	14	6	<0.001 HS
In situ urinary catheter	22	10	<0.001 HS
Congestive cardiac failure	4	6	0.22 NS
Anemia	10	44	0.4 NS

with mean duration of stay in ICU of 21+/-4.95 days. Patients with IR-PA infections suffered more number of episodes of complications related to infection. Mortality in IS-PA ventilator associated pneumonia was 20% (2/10).

Overall in hospital mortality of patients with *P. aeruginosa* infections was 13.64% (15/110): 33.3% (8/24) for IR-PA infections and 8.14% (7/86) for Imipenem sensitive *P. aeruginosa* infections (P<0.01 S).

80% (8/10) patients with IS-PA isolates recovered faster with mean duration of stay in ICU of 6.125+/-2.1 days compared to 21+/-4.95 days in IR-PA isolates (P<0.001 Highly significant). Patients with IS-PA infections had less number of complications related to infection compared to IR-PA infections (3.7 +/-1.31 versus 5.85 +/- 1.65. P< 0.001 HS).

Antimicrobial therapy with Imipenem during previous 3 weeks (58.33% versus 4.65%, P< 0.001 HS) more frequently associated with IR-PA than IS-PA infections contributing to mortality, morbidity, acquisition of IR-PA isolate and increased cost of the treatment. Most of the Predisposing risk factors were significantly associated with IR-PA than IS-PA infections.

IR-PA retained good sensitivity to Colistin, Aztreonam and Polymyxin B. Aztreonam

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

Antibiotic	IR-PA(n=24)(%)	IS-PA(n=86)(%)
Gentamycin	24 (100)	69 (80.2)
Ciprofloxacin	17 (71.83)	50(58.13)
Piperacillin	17(71.83)	65 (67.7)
Piperacillin + Tazobactam	12 (50)	58 (67.4)
Cefotaxime	24 (100)	72(83.7)
Ceftazidime	17 (71.83)	51(59.3)
Cefaperazone	22 (91.67)	57 (66.3)
Cefaperzone + Sulbactam	17 (71.83)	52(60.5)
Tobramycin	20 (83.3)	51(59.3)
Amikacin	17 (71.83)	51 (59.3)
Colistin	7 (29.2)	Not Tested
Aztreonam	4 (16.67)	Not Tested
Polymyxin B	0 (0)	Not Tested

IR-PA = Imipenem resistant *P. aeruginosa*
IS-PA = Imipenem sensitive *P. aeruginosa*

Table 5. Antibiogram types of 24 IR-PA isolates

Strain of IR-PA	Antibiogram of IR-PA	Number of infections (n)	Percentage caused (%)
1	R- Resistant to all	8	33.3
2	R- G, Pip,Pip+Tz, Ce, Cs, Cs+Sul, ToS- Cip, Cz, Ak	5	20.83
3	R- G, Cip, Ce, Cz,Cz+Sul, Cs, Cs+Sul,ToS- Pip, Pip+Tz,Ak	5	20.83
4	R- G, Cip, Ce, Cs, Cs+Sul, To, Ak S- Cz,Pip, Pip+Tz,	2	8.33
5	R- G, Pip, Pip+Tz, Ce, Cz, AkS- Cip, Cs, Cs+Sul, To	1	4.17
6	R- G, Pip, Ce, Cz, Cs, Ak, Pip+Tz, Cs+Sul	1	4.17
7	R- Pip, Pip+Tz, Cs, Cs+Sul, G, Ce, Cz, To, CipS- Ak	1	4.17
8	R –Ak, Pip, Pip+Tz, Cs, Cs+Sul, Ce, Cz, Cip, GS-To	1	4.17

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

and Polymyxin B were the drugs with least resistance, 16.67% and 0% respectively. Aztreonam was the most commonly used drug, due to higher cost and high frequency of adverse reactions seen with Polymyxin-B. Resistance to other drugs in IR-PA and IS-PA is shown in Table 4.

A total of 8 distinct antibiotic resistance profiles were observed in IR-PA isolates. Profile 1 was commonest (Resistant to all drugs except Polymyxin B, Colistin and Aztreonam) causing 33.3% (8/24) infections.

DISCUSSION

Present prospective observational study with a high incidence of Imipenem resistance (21.82%) among noscomial infections caused by *P. aeruginosa* clearly demonstrated that clinical utility of Imipenem is under threat. High incidence of Imipenem resistance is reported from this rural tertiary care hospital (just 8 years old) catering patients mainly from surrounding rural areas unlikely to be treated with Imipenem, indicating noscomial origin of these infections. However, this was an underestimation of Imipenem resistance since polymicrobial infections were excluded from the study. Higher incidence of IR-PA in the present study could be due to increasing admissions to intensive care units, of patients who were critically ill and immunocompromised and increasing use of Imipenem for empirical treatment in patients with severe infections caused by gram negative bacilli.

Varying resistance to Imipenem has been observed with *P. aeruginosa* from different hospitals across the country (India).Gladstone et.al. reported 42.8% carbapenem resistance among *P. aeruginosa* and Taneja *et.al*. have reported 36.4% in urinary tract infections caused by nonfermenters in general, with *Pseudomonas aeruginosa* as a leading pathogen[3,8]. Behera *et.al*. have reported 63.74%, Navaneet *et. al.* 12%, Hemalatha *et.al*. 16% ,[9,10,11]. But most of these studies were limited by small sample size. Incidence was observed to be over 40% in tertiary care centre at Cali. Colombia[12]. Similar and different findings of different studies probably reflect the variability in drug prescription policies, as well as the circulation of different *P. aeruginosa* strains in different settings.

Carbapenems have been used in clinical settings as a last resort for their broad-spectrum antibacterial activity and stability against various beta-lactamses produced by Gram-negative bacteria including extended-spectrum beta-lactamases. Resistance to Carbapenems is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and carbapenem hydrolyzing enzymes-Carbapenemases[13]. The global surveillance study MYSTIC pointed out that Greece, Brazil, the Czech-Republic, and Bulgaria possess the highest individual resistance to carbapenems, probably indicating high consumption of Carbapenems. Compared to Imipenem, however meropenem is more potent and is active against up to one third of Imipenem-resistant strains, which indicates that a considerable percent of the strains should have lost Opr D porin, which is influential mainly at Imipenem[14].

Distribution of IR-PA infections was not uniform in our hospital. Most of the isolates (80.65%) were from ICUs than from general wards (19.35%). Four of the six IR-PA from general wards were from patients shifted from ICUs. Highest incidence of IR-PA in the present study was from MICU and specimen wise distribution shows highest incidence from blood. Gupta et. al. have reported higher incidence of IR-PA from ICU patients compared to non-ICU patients (50% Vs 31.9%)[15]. ICU is a "MELTING POT" for dissemination of IR-PA isolates since most of the isolates were from ICU patients or related to ICU admission. *P. aeruginosa* and other nonfermenters are very important noscomial pathogens in ICUs as these strains may often cause outbreaks[3]. Absence of IR-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-PA infections was found to be just short of endemicity. The occurrence of IR-PA 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 management. 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. Higher incidence of IR-MBLP-PA infections in the present study was due to rapid emergence, spread from critical care units of our hospital. There exists an impending threat of Imipenem resistance in other gram negative nosocomial infections since pseudomonas can transfer carbapenem resistance genes through plasmids to Enterobacteriaceae, probably within a clinical environment there by increasing the burden of Imipenem resistance in nosocomial pathogens.

Previous antibiotic therapy with Imipenem was significantly associated with acquisition of IR-PA isolates (14/24 versus 4/86 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[16,17]. Although treatment with Imipenem could more often result in the emergence of resistant *P. aeruginosa* than treatments with other antipseudomonal agents, this tendency may not translate into higher prevalence of imipenem resistance among hospital isolates[17]. However, emergence and persistence of IR-PA at our hospital suggests different mechanisms of resistance namely production of metallo-beta-lactamses, strains of which are known to spread among different areas of the hospital. between hospitals and into community. Although most carbapenem resistance in *P. aeruginosa* remains as a result of porin loss, there must be concern about the growing number of outbreaks, some of them large and protracted, caused by IMP, VIM and SPM metallo-carbapenamases[18]. Therefore the present study necessitates inquiry into the metallo-beta-lactamase production in nosocomial *P. aeruginosa* isolates.

Newer carbapenems such as Ertapenem and Doripenem could offer pharmacological advantages over Imipenem and Meropenem in *P. aeruginosa* nosocomial infections but do little to overcome resistance to these older carbapenems[18].

Increased mortality in IR-PA infections was associated with more frequent isolation of PAN DRUG resistant isolates (strain 1)among IR-PA compared to IS-PA (8 versus 0) and multidrug resistant isolates (16 versus 4). Clinicians were practically left with no option for treating patients with PAN DRUG resistant IR-PA infections. Gladstone *et. al*. have reported higher mortality and morbidity among *P. aeruginosa* and other nonfermenters due to multidrug resistant isolates limiting the therapeutic options in patients with severe underlying diseases[3]. Diabetes mellitus was significantly associated with IR-PA in the present study. Varaiya *et. al*. have reported 26.5% mortality among patients with Diabetes mellitus and malignancy in nosocomial infections caused by *P. aeruginosa*[19].

Worldwide emergence of MDR nosocomial clones has added significantly to ominous prognosis of *P. aeruginosa* infections. MDR *P. aeruginosa* infections are associated with three fold higher rate of mortality, a ninefold higher bacteremia, a twofold increase in the length of hospital stay with a considerable increase in the cost of treatment. Because of versatility and large genome, various resistance mechanisms may be present simultaneously, causing cross resistance to several antipseudomonal agents. The increasing resistance rate of *P. aeruginosa* strains to several antibiotics are expanding globally as per the reports of NNIS (National Nosocomial Infection Surveillance) programme. Globally, isolates of *P. aeruginosa* posses the highest individual resistance rate to Imipenem, probably indicating high consumption rate of Imipenem[13].

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[16].

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. VAP due to IR-PA significantly increases mortality since best diagnostic approach to therapy, rotational therapy and unconventional approaches to

antimicrobial therapy remain uncertain[16]. VAP due to IR-PA was an independent risk factor for mortality and morbidity in the current study.

In contrast to this, survival of 16 patients with IR-PA from ,nosocomial tacheobronchitis, post operative wound infections, burns wound infections (< 20%) and urinary tract infection signifies the role of less severe underlying disease as an important predictor of good prognosis.

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-PA infections with attended MDR and PDR isolates will worsen, while the era of "The end of Antipseudomonal antibiotics" will become a nosocomial nightmare[13].

This study documents higher virulence of IR-PA isolates due to rapid downhill course to death with a short mean duration of stay in ICU till death and higher frequency of complications related to infections. Although with higher virulence, 33.3% of IR-PA infections did not result in mortality and on the contrary 4 patients with 50% burns with IS-PA rapidly progressed to death. These findings yet again underscore the role of severe underlying disease and predisposing risk factors contributing significantly to mortality and morbidity. Similar studies are not available for comparison after review of literature.

Diabetes mellitus, Long term IV cannulation, admission to ICU in preceding 3 weeks, COPD, Smoking, Previous antibiotic therapy other than Imipenem, treatment with steroids and in situ Foley's catheter were significantly associated with IR-PA than IS-PA, significantly contributing to mortality , morbidity and increased health care cost.

Although, partially mediated by higher virulence, mortality and morbidity in IR-PA infections was associated with severe underlying disease, previous antibiotic therapy with Imipenem, MDR and PDR isolates in patients with multiple predisposing risk factors. Though predisposing risk factors were analyzed objectively by appropriate statistical test (student "t" test) their role in a particular patient was more or less subjective. Impact of IR-PA isolate on mortality and morbidity in an index patient could be assessed in the background of these risk factors prevailing in a patient at the time of isolation of IR-PA.

Majority of the IR—PA infections in this study were caused by antibiotic resistance strain 1. Clonal relation and dissemination of IR-PA strains could not be assessed as molecular typing was not done.

Timely identification of increased isolations of IR-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-PA isolates within a hospital.

Conclusions of the study

1. High incidence of Imipenem resistant P.aeruginosa from a new, rural tertiary care hospital indicates emergence, persistence and spread within the hospital. These findings can be generalized to other tertiary care hospitals with similar conditions
2. Imipenem resistant *P. aeruginosa* (IR-PA) cause significantly higher mortality compared to Imipenem sensitive *P. aeruginosa* infections (IS-PA)
3. Attributable mortality and morbidity in IR-PA infections, is partially mediated by IR-PA isolates, severity of underlying disease, predisposing risk factors, Multidrug resistance, Pan drug resistance, and increased virulence, making IR-PA isolate, a nosocomially successful and difficult to treat pathogen
4. Patients in whom Imipenem is selected as antipseudomonal antibiotic, the potential for emergence of IR-PA strains should be anticipated, and in appropriate circumstance, routine culture and screening for Imipenem resistance should be performed to detect the emergence of IR-PA strains

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