Study of Resistance Pattern in Mucoid and Non Mucoid Isolates of *Pseudomonas aeruginosa* from Lower Respiratory Tract Specimens in a Tertiary Care Centre of Western U.P.

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ABSTRACT

Introduction: Respiratory tract infections are a major cause of ambulatory visits to the family practitioners .However, increase in antibiotic resistant strains of bacteria has complicated the use of empiric therapy of this common human disease. Among the Gram negative bacilli which are the commonest pathogen of LRTI, *Pseudomonas aeruginosa* is the most challenging, because of its high rate of resistance to antimicrobial agent. Objectives: To obtain a comprehensive insight into the different resistant types: Multi drug resistant, Extensively drug resistant, Carbapenem Resistant, and MBL producing *Pseudomonas aeruginosa* isolated from lower respiratory tract specimens and antibiotic susceptibility differences between its mucoid and non mucoid colony types based on colony morphology.

Material and Methods: A total of 926 lower respiratory tract samples consisting of sputum,pleural fluid,endotracheal aspirates,Bronchoalveolar lavage from patients of all age and sex , suggestive of LRTI were included . Following Direct Gram staining and culture, the organisms were isolated and Pseudomonas aeruginosa among them were identified by standard biochemical tests. The different types of colony morphologies of Pseudomonas aeruginosa and the antimicrobial susceptibility differences amongst the different colony types were statistically analysed.

Results: A total 175 (*18.8%) Pseudomonas* were isolated from different Lower respiratory specimen Out of these, only 103 *Pseudomonas aeruginosa* were found to be clinically significant with 84.5% non mucoid and 11.4% mucoid colony types. The mucoid colony types showed high resistance to Cefepime (35%), followed by Ceftazidime (20%) and Amikacin (15%).

Conclusion: The high rate of MDR and XDR *Pseudomonas aeruginosa* also resistant to Carbapenems from this region reveals a frightening scenario.As molecular methods are not available in majority of resource constrained laboratories of India, the phenotypic methods should be regularly performed to detect the various beta-lactamases, besides strict infection control practices.

Keywords: MBL, Carbapenem Resistant, MDR and XDR *Pseudomonas aeruginosa*

INTRODUCTION

Lower respiratory tract infections are common human diseases requiring consultation and hospitalization. The morbidity and mortality may range from minor self limiting illness to potentially life threatening infections. The bacteriological profiles of the LRTIs are different in different countries, which may vary with age sex, and season within the same country. They are commonly the first infection to occur post birth and pneumonia is quite often the final illness to occur before death.[1] A variety of organisms are usually implicated in their aetiologies, the most common being the Gram negative bacilli[2]

Pseudomonas aeruginosa are one of the important causes of nosocomial and community acquired LRTI. Resistance of Pseudomonas aeruginosa to a wide range of Antibiotics may result in increased morbidity and mortality. The colony morphology of *P. aeruginosa* is also varied along with differences in their antimicrobial susceptibility pattern,the most common being mucoid and non mucoid ones.

P. aeruginosa may be a primary pathogen of the respiratory tract or merely a colonizer in patients of prolonged hospital stay with intubation and tracheostomy done ,which may advance to life threatening infections or may remain inconsequential.[3] Thus identification of *Pseudomonas aeruginosa* as primary pathogen or a colonizer becomes important and specific microbiological investigations are required to enable the clinician to choose appropriate antibiotic and prevent prophylactic and irrational administration of antibiotic.

In recent years, a significant increase in the prevalence of multidrug resistant *P. aeruginosa* (MDRPA) has been noticed.For treatment of MDR-PA infections, carbapenems, especially Imipenem and Meropenem are used. However, the prevalence of imipenem resistance to *P. aeruginosa* has been increasing worldwide. One of the reasons of resistance is the production of metallo-beta-lactamases. The presence of this mechanism can lead to treatment failure in carbapenem therapy of *P. aeruginosa* infections.[4] MBLs are

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a class B type of betalactamases that require bivalent metal ions, usually zinc for their activity.[5] Metallo-β- lactamases (MBL) have emerged as one of the most troublesome resistance mechanisms owing to their capacity to hydrolyze all β-lactams, including carbapenems. The occurrence of metallo β-lactamase producing *Pseudomonas aeruginosa* in a hospital environment poses not only a therapeutic problem but is also a serious concern for infection control management.

Thus the present study was undertaken to detect Multi drug resistant, Extensively drug resistant, Carbapenem Resistant,and MBL producing *Pseudomonas aeruginosa* isolated from lower respiratory tract specimens from Rohillkhand region .The changing trends in the antibiogram of its most common colony types mucoid and Non mucoid were also analysed.

MATERIAL AND METHODS

This study was a cross sectional study conducted in the Department of Microbiology, Rohilkhand Medical College and Hospital, Bareilly from November 2016 to October 2017 after approval of Ethical committee. A total of 926 lower respiratory tract specimens which included 808 sputum, 95 Pleural fluid, 14 Broncho-alveolar lavage and 8 Endotracheal aspirates from all age and sex groups were included in the study. The specimen were collected according to established guidelines with aseptic precautions. All the specimens were subjected to direct Gram's staining from the specimen, and culture on Blood agar and Mac conkey agar. *Pseudomonas* were identified by their colony morphology on the respective media, motility test and biochemicals like oxidase test, Triple sugar iron and OF test. Pseudomonas aeruginosa were further identified by demonstration of blue phenazine pigment Pyocyanin or yellow green pigment pyoverdin giving the characteristic blue green appearance of cultures, Arginine dihydrolase test and growth at 42ºC. Presence of fever clinically and presence of Leukocytosis with Polymorphonuclear cells in Grams staining were also recorded and interpreted in concordance to culture to differentiate Pseudomonas aeruginosa as colonizers or primary pathogens.

All the clinically significant *Pseudomonas* aeruginosa isolates were subjected to Antimicrobial susceptibility testing by Kirby baeur disc diffusion method as per the protocol described by CLSI (M100, 28th 38-40)[6] using the following Antibiotic Discs namely Amikacin, Ciprofloxacin, Gentamicin, Ceftazidime, Cefepime, Piperacillin, Piperacilline/tazobactam, Imipenem, Meropenem, Doripenem, Azteronam, Colistin. The Antibiotic susceptibility differences between the mucoid and non mucoid isolates of Pseudomonas aeruginosa was also recorded.

Further , all the isolates of Pseudomonas aeruginosa found to be resistant to Carbapenems Imipenem, Meropenem, Doripenem with a zone size of less than 15 mm were screened for Carbapenemase production and detection of Metallo beta lactamase by Modified Hodge test, Imipenem and Imipenem EDTA Combined disc Test as described by Yong et al[7] and MBL E-test using EM078 Imipenem with and without EDTA Ezy MICTM Strip as described below:

Imipenem –EDTA Combined disc test (CDT)

The test isolates along with standard control strains(opacity adjusted to 0.5 McFarland opacity standard) were lawn cultured on Mueller –Hinton agar plate as recommended by CLSI. After drying, 10µg Imipenem discs and Imipenem-EDTA 10\750 mcg disc were placed on the lawn culture with 20 mm distance from centre to centre of the discs and incubated over night.Isolates showing >7mm increase in the inhibition zone size of Imipenem-EDTA disc than the Imipenem disc alone were considered as MBL producers.[7] (Fig 2).

Modified Hodge Test (MHT)

Modified Hodge test is a screening test which helps in detection of Carbapenemases.[8] Escherichia coli ATCC 25922 (an indicator organism sensitive to carbapenems) was cultured in peptone water to achieve 0.5 McFarland opacity standard) and lawn cultured on Mueller-Hinton agar plate using sterile cotton swab. After drying ,10µg Imipenem disc was placed at the centre of the plate on the lawn culture and an overnight growth of test strain was heavily streaked from the edge of the Imipenem disc outwards,to the periphery of the plate in four different directions. The plates were incubated at 37ºC overnight. The presence of a distorted zone (Clover leaf shaped zone of inhibition) was considered as positive test for Carbapenemase production.

MBL E-Test: All the Imipenem resistant isolates were subjected to E-Test to detect minimum Inhibitory concentration(MIC) ratio and to confirm MBL production.[9] Imipenem with and without EDTA Ezy MICTM Strip(Hi Media) were used for MBL detection .The E-test MBL strip is coated with mixture of Imipenem + EDTA (1-64 mcg/ml) and Imipenem (4-256 mcg/ml) on a single strip in a concentration gradient manner. When the ratio of the value obtained for Imipenem (IPM): the value of IPM + EDTA was more than to or 8 or if the zone was observed on the side coated with IPM+EDTA and no zone was observed on the opposite side coated with IPM, the culture was interpreted as MBL positive (Fig 3).

RESULTS

From a total of 926 Lower respiratory tract specimens 175 (*18.8%) Pseudomonas* were isolated, 140 (80%) from sputum and 28 (16%) from pleural fluid ,5 (2.85%) from BAL and 2 (1.14%) from E.T tube. The other microorganisms isolated included Klebsiella, Staphylococcus aureus, Citrobacter, Candida etc. Out of these, only 145 were confirmed to be *Pseudomonas aeruginosa.* Further, in 42 out of them colony count was either insignificant or mixed growth with other gram negative bacilli viz E. coli or Klebsiella was found. On clinical analysis these patients were found to have prolonged duration of hospital stay without any fever or leukocytosis. Their sputum on Gram's staining did not also show polymorphonuclear leukocytes (PMN) nor the chest radiograph showed a new infiltrate or the expansion of a pre-

P.aeruginosa n=103	Number	OPD	IPD	Clinical finding Either or all
Sputum	87	15	72	Fever, leukocytosis, PMN in Gram's stain in concordance with culture
Pleural fluid	13	0	13	Fever, Leukocytosis, PMN in Gram's stain with chest X ray showing infiltrates
BAL	3	0	3	Fever, Leukocytosis, PMN in Gram's stain, pure growth of *Pseudomonas* on culture
ET	nil			
Total	103			

Table-1: Distribution of clinically significant Pseudomonas aeruginosa from different respiratory tract specimens.

Pseudomonas N=175	Non mucoid	Mucoid	Small colony variant	Dwarf colonies	Rugose	Pepper corn
Total %	148 (84.5%)	20 (11.4%)	5 (2.8%)	2 (1.1%)	0	0

Table-2: Showing total number of Mucoid, non-mucoid and other colony types of Pseudomonas

Antibiotics	Sensitive		Intermediate		Resistant		
	Non Mucoid Number%	Mucoid Number%	Non Mucoid	Mucoid	Non mucoid	Mucoid	P value
Amikacin	43 (51.8%)	16 (80%)	6 (7.2%)	1 (5%)	34 (40.9%)	3 (15%)	.068
Gentamicin	34 (40.9%)	15 (75%)	8 (9.6%)	2 (10%)	41 (49.3%)	3 (15%)	.015
Ciprofloxacin	40 (48.1%)	16 (80%)	6 (7.2%)	1 (5%)	37 (44.5%)	3(15%)	.034
Cefepime	24 (28.9%)	11(55%)	5(6%)	2(10%)	54(65%)	7(35%)	.048
Ceftazidime	31 (37.34%)	15 (75%)	7 (8.4%)	1 (5%)	45 (54.2%)	4 (20%)	.009
Piperacillin	54 (65%)	17 (85%)	7 (8.4%)	0	22 (26.5%)	3 (15%)	.174
Piperacillin/Tazobactam	71 (85.5%)	20 (100%)	0	0	12 (14.4%)	0	.070
Imipenem	49 (59%)	15 (75%)	6 (7.2%)	0	28 (33.7%)	5 (25%)	.288
Meropenem	53 (63.8%)	19(95%)	3(3.6%)	0	27(32.5%)	1(5%)	.024
Ertapenem	55 (66.2%)	20	2	0	26	0	.010
Aztreonam	65 (78.3%)	17	3	0	15	3	.637
Colistin	78 (93.9%)	20	0	0	5	0	.260

Table-3: Antibiotic susceptibility pattern (AST) of n= 103 isolates of mucoid and mucoid isolates of Pseudomonas aeruginosa (number of strains%)

Respiratory sample n=33	Total Imipenem resistant	Modified Hodge test positive	Imp –EDTA combined disc test positive	E –Test Positive
Sputum	24	16	13	11
Pleural fluid	7	5	2	2
Bronchoalveolar lavage	2	2	1	1
Total	33	23	16	14

Table-4: Distribution of Imipenem resistant isolates and their results when subjected to Modified Hodge test, Imipenem EDTA combined disc test and E test.

n=103	No. of Isolates	Percentage
MDR	53	51.4%
XDR	15	14.5%
MBL	14	13.5%
CRPA* due to mechanism other than MBL	9	8.7%
*Carbapenem resistant Pseudomonas aeruginosa		

Table-5: Categorization of Pseudomonas aeruginosa into various resistant phenotypes.

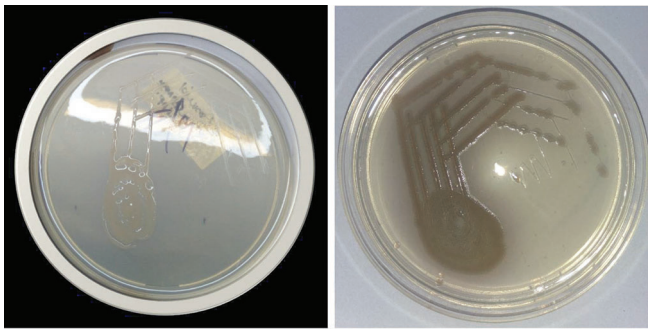

Figure-1: Showing mucoid (left) and non mucoid (right) colonies of Pseudomonas on Nutrient Agar

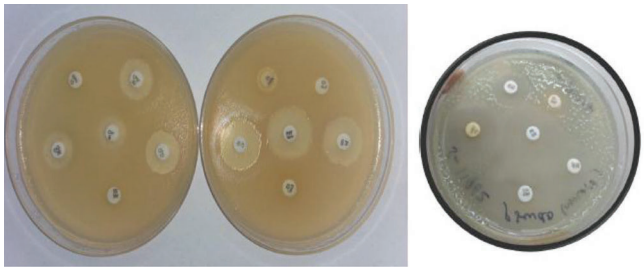

Figure-2: Showing differences in Antimicrobial susceptibility pattern of Non Mucoid and Mucoid isolates of *Pseudomonas aseuginosa*

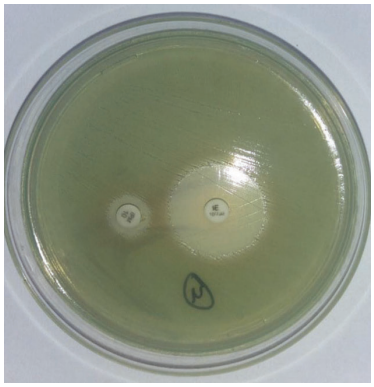

Figure-3: Combined disc test using Imipenem and imipenem-EDTA. Imipenem + EDTA disc (on the right) produce ≥ 7 mm larger zone of inhibition than the imipenem disc (on the left)

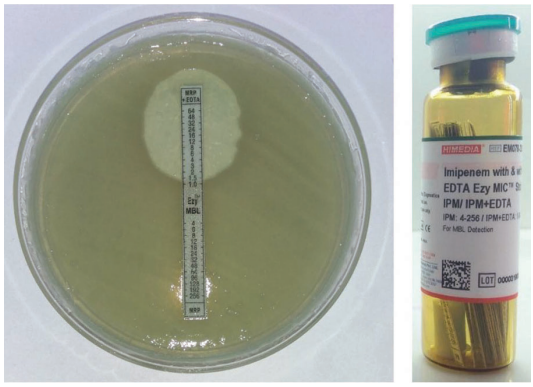

Figure-4: Showing MBL producing *Pseudomonas aeruginosa* by E- test

existing infiltrate. Thus, only 103 *Pseudomonas* aeruginosa were found to be clinically significant. The distribution of 103 clinically significant Pseudomonas aeruginosa is shown in table 1. The total number of mucoid , non mucoid and other colony types of Pseudomonas aruginosa is shown in table 2.

Antimicrobial susceptibility pattern of mucoid and non-mucoid *P. aeruginosa* against 12 different tested antibiotic was determined (table 3). Among mucoid isolates, high resistance to Cefepime (35%), followed by Ceftazidime (20%) and Amikacin (15%) was observed where as nonmucoid isolates showed high resistance to Cefepime (65%), Ceftazidime (54.2%), Gentamicin (49.3%), Ciprofloxacin (44.5%), Amikacin (40.9%), Imipenem (33.7%), Meropenem (32.5%), and Ertapenem (31.3%). The difference between the mucoid and non mucoid group of sensitive, Intermediate and resistant for Gentamycin, Ciprofloxacin, β-lactam drugs like Cefepime and Ceftazidime and Carbapenems like Meropenem and Ertapenem were found to be statistically significant ($p< 0.05$).

33 isolates of *Pseudomonas aeruginosa* also showed zone of inhibition for Imipenem less than or equal to 15 mm which is considered resistant for imipenem by Kirby-Bauer disc diffusion method according to CLSI guidelines. The sample wise distribution of these 33 isolates and their results when subjected to Modified Hodge test, Imipenem EDTA combined disc test and E test is summarized in table 4. On the basis of MBL E-test which is considered to be a sensitive method for detection of MBL with reported 100% accuracy by Khosravi et al[10] total 14 (13.5%) *Pseudomonas* aeruginosa from our institute were found to be MBL. The different resistant phenotypes of Pseudomonas aeruginosa isolated in the present study have been tabulated in table 5.

DISCUSSION

Lower Respiratory tract infections are perhaps the most frequently reported infections of human being. These infections are usually mild, transient lasting and self-limiting due to which many infected individuals tend to disregard them but the spectrum of disease ranges from mild mucosal colonization to acute bronchitis or acute exacerbation of chronic bronchitis, chronic obstructive pulmonary disease and severe community acquired pneumonia.[11,12]

According to Global burden of Disease study 2015, Lower respiratory tract infections caused about 529381.1 deaths in India and the burden of the disease in terms of DALYs (Disability Adjusted Life Years) lost was 121.15 million.[13]

The etiological agents of LRTIs cannot be determined clinically and differ from area to area. Gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus pneumonia* as well as Gram negative bacteria such as *Haemophilus influenzae*, *Pseudomonas, Acinetobacter, and Klebsiella species* are recovered from LRTIs.[14,15]

Pseudomonas aeruginosa is an opportunistic pathogen and the predominant causative agent in nosocomial LRT infections. It is also considered as the most challenging pathogen worldwide, because of its high rate of resistance to antimicrobial agent[16] *Pseudomonas aeruginosa* is inherently resistant to many antibiotics and disinfectants, including antipseudomonal penicillins, ceftazidime, carbapenems,

aminoglycosides, and ciprofloxacin.

Multidrug-resistant *P. aeruginosa* (MDR-PA) is a matter of great concern as it not only causes severe and fatal infections but also increases the length of hospital stay, resulting in increased treatment costs.[17] Carbapenems are effective antibiotics against MDR-PA infections. However, their use in the management of infection is threatened by the development of carbapenem-resistant *P. aeruginosa* (CRPA) strains.[18,19] Resistance to the carbapenems in *P. aeruginosa* is often caused by impermeability through alteration or loss of the porin OprD, increased expression of an efflux pump, or the production of class B metallo-β-lactamases (MBLs).[20,21,22] MBL producing *P. aeruginosa* have emerged as one of the most feared resistance mechanisms. MBL production in *P. aeruginosa* can be detected by molecular methods and phenotypic methods. In molecular methods, polymerase chain reaction (PCR), DNA probes, cloning and sequencing can be done to detect MBL positive genes. These methods are highly accurate and reliable, but they are available only in reference laboratories. The phenotypic methods of MBL production are based on the ability of metal chelators such as EDTA and thiol-based compounds to inhibit the activity of MBL. The common methods are modified Hodge test (MHT), combined disc diffusion test using imipenem and EDTA, and MBL E- test .

The present study is an attempt to provide an insight into the various resistant types of Pseudomonas aeruginosa including MBL and study the differences in antibiogram on the basis of colony morphology.

The infection rate of *Pseudomonas* aeruginosa amongst the patients suffering from different LRTI was found to be 11.1% in the present study.

P. aeruginosa shows a variety of colony types that are important for epidemiological purposes as mucoid and nonmucoid phenotypes of *Pseudomonas aeruginosa* also have apparent differences in their antimicrobial susceptibility pattern (Fig 1). In the present study, out of total 175 *Pseudomonas* isolated 148 (84.5%) were nonmucoid, 20 (11.4%) mucoid type, 5(2.8%) small colony variant and 2 (1.1%) dwarf colonies. Other colony types likes pepper corn and rugose mentioned in literature were not isolated during the study period. Owlia. P et al[23] from Tehran, found 50 (50%) nonmucoid type and 50 (50%) mucoid type of *Pseudomonas aeruginosa* out of 100 sample. Prem P Mishra and Ved Prakash[24] from our institute in 2016, isolated 57 (79.87%) nonmucoid strains and 15 (20.83%) mucoid stains of *Pseudomonas aeruginosa* out of 72 respiratory samples.

We observed that the more prevalent non mucoid variant was alarmingly resistant to different tested antibiotics. Where as mucoid isolates showed high resistance to cefepime (35%) followed by Ceftazidime (20%) and Amikacin (15%) , the nonmucoid isolates showed high resistance to Cefepime (65%), Ceftazidime (54.2%), Gentamicin (49.3%), Ciprofloxacin (44.5%), Amikacin (40.9%), Imipenem (33.7%), Meropenem (32.5%), and Ertapenem (31.3%) (Fig 2). Thus, it was found that mucoid isolates were more susceptible to antibiotics as compared to nonmucoid ones.These findings were consistent with the findings of Srifuengfung[25] and Shawar et al.[26] The difference between the mucoid and nonmucoid group of sensitive, Intermediate and resistant for Gentamycin, Ciprofloxacin, β-lactam drugs like Cefepime and Ceftazidime and Carbapenems like Meropenem and Ertapenem were found to be statistically significant ($p< 0.05$), while no significant difference was observed among mucoid and nonmucoid strains to other tested Antibiotics. (P value> 0.05).

According to Ciofu et al[27] the relatively higher resistance pattern seen among nonmucoid strains may be due to their higher exposure to antibiotic selective pressure than mucoid type as the alginate hyper producing mucoid phenotypes are generally protected within the multiple layers of biofilm.

In our study, 33 (32%) strains of *Pseudomonas aeruginosa* resistant to Carbapenems notably Imipenem, Meropenem and Ertapenem were also isolated by Kirby-Bauer disc diffusion method. Out of these 24 (23.3%) were from sputum 7 (6.8%) from pleural fluid and 2 (1.9%) from bronchoalveolar lavage. Carbapenem group of antibiotics play a vital role in the management of nosocomial infections due to gram negative organisms owing to their broad spectrum of activity and have a unique structure that is defined by a carbapenem coupled to a β-lactam ring which confers protection against most β-lactamases.[28] However, the use of Carbapenems has been hampered by the emergence of strains that produce metallobeta- lactamase, an enzyme that is able to hydrolyze and inactivate this class of antibiotics. Screening of Carbapenemase producers among Carbapenem resistant *Pseudomonas aeruginosa* is important for appropriate and judicious use of antibiotic therapy and prevent the development of nosocomial outbreaks.

Different studies have reported the use of methods like Imipenem-EDTA double disc synergy test, Imipenem EDTA combined disc test,Modified Hodge test and MBL E Test according to which MBL production ranged from 7% to 65%.[9] As molecular detection of Carbapenemase genes is not only costly but also requires a high degree of expertise available only in specialized laboratories,simple and reliable tests are needed to detect MBL producers.

In the absence of any guidelines for phenotypic detection of MBL, it was done by using three phenotypic methods Modified Hodge test, Imipenem –Imipenem EDTA combined disc test and E-test in our study. MHT ,though a significant test for screening of carbapenenase activity, cannot distinguish MBL carbapenemases from non MBL carbapepemases. Several authors like Franklin et al[29] and Sinha et al[30] have also concluded MHT as least sensitive and specific for MBL detection but a good screening tool.

CDT on the other hand, has been reported to be sensitive and specific by several authors like Behera et al[31], Murgan et al[32], Varaiya et al[33], for detection of MBL production as compared to other tests. CDT for MBL production is simple to perform and materials used are cost effective, non toxic and easily available which makes it a efficient screening tool for MBL in routine clinical laboratories.

MBL E-test is a sensitive method for detection of MBL in *P.*

aeruginosa. The E-test, based on a combination of β-lactam substrate and a β-lactam/ MBL inhibitor, is specifically designed to detect as many clinically relevant MBL as possible. Khosravi et al[10] have reported 100% accuracy of MBL E-test with PCR for the detection of MBL production. We observed that, out of 33 Imipenem resistant isolates by Kirby Baeur Disc Diffusion method 23 were positive by Modified Hodge Test, 16 by Combined Disc test and 14 by MBL E-test. Thus,13.5% Pseudomonas aeruginosa were concluded to be MBL.

The 2 strains of Imipenem resistant *Pseudomonas aeruginosa* positive by MBL E test but negative by combined disc test may be due to use of only one test i.e. combined disc test using Imipenem/EDTA on our part. If it were possible on our part to use Combined disc test along with Double disc synergy test, or use of other chelators like mercaptoacetic acid, these two tests would have better correlated.

Pseudomonas aeruginosa also showed resistance to many other classes of antibiotics including Aminoglycosides, beta-lactam drugs (cefepime, ceftazidime, piperacillin) and Ciprofloxacin. This high level of resistance is attributable to the multiple intrinsic resistance mechanisms that *P. aeruginosa* may express, including beta-lactamase production, efflux- mediated porin-related resistance, and target site modification. These mechanism are often present in coexistence with genes encoding drug resistance to other antibiotics on the plasmid which encode ESBL and MBL. Overall, 53 (51.4%) isolates were found to be MDR in this study,which is defined as isolates resistant to at least 3 classes of drugs in antipseudomonal cephalosporin, carbapenem, aminoglycosides and fluoroquinolones. MDR is a growing clinical problem and is also recognized as a threat to public health in causing significant morbidity and mortality and increase economic burden which stems from the misuse of antibiotics. The percentage of MDR *Pseudomonas aeruginosa* in India ranges from 11.36% reported by Siti Nur Atiquah et al[34] to 91.6% reported by Panaranjothi et al.[35] Other authors like Senthamarai[36] and his co-workers have reported 41.35% where as Biswal et al[37] from Safdarjang hospital new Delhi have reported 36.2% of MDR Pseudomonas aeruginosa.

14.5% of *Pseudomonas* aeruginosa in the present study were also found to be Extensively Drug Resistance (XDR) which is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories according to A.P Magiorakos et al[38]

Moreover, out of 14 strains of MBL producing *Pseudomonas* aeruginosa,13 (92.8%) were also XDR *Pseudomonas aeruginosa,* sensitive only to Colistin (100%), Aztreonam (92.8%) and Piperacillin (35.7%) and Piperacillin\ Tazobactam (78.5%) combination. These highly resistant gram negative MDROs reflect a threatening picture considering the limited number of treatment option left and limited number of new antimicrobial agents in development. Laboratory screening test for detection of carbapenem resistant organisms by Modified Hodge test showed 9 (8.7%) isolates of *Pseudomonas aeruginosa* to be carbapenem resistant by mechanism other that MBL production in our study which could not be further identified due to lack of molecular laboratory in our hospital setting. These 8.7% Carbapenem Resistant Pseudomonas aeruginosa may be class A carbapenemases IMI-1 (Imipenem bete hydrolyzing enzyme 1) or Class D carbapenemases of the OXA enzyme type particularly found in non-fermenters like *Pseudomonas aeruginosa* and *Acinetobacter species.* Noyal et al[39] from JIPMER, Puducherry and Basak et al[40] from JNMC, Wardha have isolated 28.1% and 11.4% carbapenemase producing Pseudomonas aeruginosa respectively by the above method.

CONCLUSION

Thus, it is well evident from this study and current data that MBLs and carbapenemases are major threat for the 21st Century in the field of microbial drug resistance. As molecular methods are not available in majority of resource constrained laboratories of India, the phenotypic methods should be regularly performed to detect the various beta-lactamases. These phenotypic methods are not only easy to perform and economical but can also discriminate among the various beta lactamases which even the automated systems fail to detect. Strict infection control practices, judicious use of antibiotics, early detection of the MBL carriage, all will together help in the longevity of the carbapenems, which are the last resort antibiotic

REFERENCES

1. Sarmah N, Sarmah A, Das DK. A study on the Microbiological Profile of Respiratory Tract Infection (RTI) in patients attending Gauhati Medical College and Hospital.Annals of International Medical and Dental Sciences, vol(2), Issue (5).
2. Elumalai Arthi, Raj M Anita, V. Abarna, R. Bagyalakshami, Reddy S. Study of Gram Negative Bacterial isolates from Lower Respiratory Tract Infections (LRTI) and their Antibiogram Pattern in a Tertiary care Hospital in South India. JMSCR 2016;4:14066-14070.
3. Saxena Shivani, Bannerjee G., Garg Rajiv, Singh Mastan, Verma S.K, Kushwaha Ras. Bacterial colonization in patients with lower respiratory tract specimens:demographic profile and microbiological pattern.International Journal of Medical science and Public Health 2015; vol 4, Issue 11.
4. Laupland KB, Parkins MD, Church DL, et al. Population-based epidemiological study of infections caused by carbapenem-resistant *Pseudomonas aeruginosa* in the Calgary Health Region: Importance of metallo-β-lactamase (MBL)- producing strains. J Infect Dis. 2005;192:1606–1612.
5. Bush K. beta-Lactamases of increasing clinical importance. Curr Pharm Des 1999;5:839-45.
6. Clinical and Laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing, 28th edition. M100, p 38-40
7. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y .Imipenem –EDTA disc method for differentiation of metallo beta lactamases producing clinical isolates of Pseudomonas spp and Acinetobacter spp.J Cli Microbiol

2002;40:3798-801.
8. Amudhan SM, Sekar U, Arunagiri K, Sekar B. OXA Beta-lactamase-mediated carbapenem resistance in Acinetobacter baumannii. Indian J Med Microbiol. 2011;29:243-5.
9. Shivaprasad A, Antony B, Shenoy P. Comparative Evaluation of four phenotypic Tests for detection of Metallo-ß-Lactamase and Carbapenemase Production in Acinetobacter baumannii.Journal of Clinical and Diagnostic Research. 2014;8:DC05-DC08.
10. Khosravi Y, Loke MF, Chua EG, Tay ST, Vadivelu J. Phenotypic detection of metallo-ß-lactamase in imipenem-resistant Pseudomonas aeruginosa. Scientific World Journal 2012;2012:654939.
11. Ndip RN, Ntiege EA, Ndip LM, Nkwelang G, Aoachere JF, Akenji TN. Antimicrobial resistance of bacterial agents of the upper respiratory tract of school children in Buea, Cameroon. J Health Popul Nutr. 2008; 26:397-404.
12. Alter SJ, Vidwan NK, Sobande PO, Omoloja A, Bennett JS. Common childhood bacterial infections. Curr Probl Pediatr Adolesc Health Care. 2011; 41:256-83.
13. Troeger. C et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. GBD 2015 LRI Collaborators.
14. Ozyilmaz E, Akan OA, Gulhan M, Ahmad K, Nagatake T. Major bacteria of community-acquired respiratory tract infections in Turkey. Jpn J Infect Dis. 2005; 58:50-2.
15. Erling V, Jalil F, Hanson LA, Zaman S. The impact of climate on the prevalence of respiratory tract infection in early childhood in Lahore, Pakistan. J Pub Health 1999; 21:331-9.
16. Fatima A, Nagvi SB, Khaliq SA, Perveen S, Jabeen S. Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosa isolated from patients of lower respiratory tract infections. Springerplus 2012; 1:70.
17. Davane M, Suryawanshi N, Pichare A, Nagoba BS. Pseudomonas aeruginosa from hospital environment. J Microbiol Infect Dis 2014;4:42-3.
18. Kateete DP, Nakanjako R, Namugenyi J, Erume J, Joloba ML, Najjuka CF, et al. Carbapenem resistant Pseudomonas aeruginosa and Acinetobacter baumannii at Mulago hospital in Kampala, Uganda (2007-2009). Springerplus 2016;5:1308.
19. Liakopoulos A, Mavroidi A, Katsifas EA, Theodosiou A, Karagouni AD, Miriagou V, et al. Carbapenemase-producing Pseudomonas aeruginosa from central Greece: Molecular epidemiology and genetic analysis of class I integrons. BMC Infect Dis 2013;13:505.
20. Rojo-Bezares B, Cavalié L, Dubois D, Oswald E, Torres C, Sáenz Y, et al. Characterization of carbapenem resistance mechanisms and integrons in Pseudomonas aeruginosa strains from blood samples in a French hospital. J Med Microbiol 2016; 65:311-9.
21. Lee JY, Ko KS. OprD mutations and inactivation, expression of efflux pumps and ampC, and metallo-β-lactamases in carbapenem-resistant Pseudomonas aeruginosa isolates from South Korea. Int J Antimicrob Agents 2012;40:168-72.
22. Sachdeva R, Sharma B, Sharma R. Evaluation of different phenotypic tests for detection of metallo-β-lactamases in imipenem-resistant Pseudomonas aeruginosa. Journal of Laboratory Physicians 2017;9:249- 253.
23. Owlia P, Nosrati, Alaghehbandan R, Lari AR. Antimicrobial susceptibility difference among mucoid and non-mucoid Pseudomonas aeruginosa isolates. GSM Hygine and Infection Control 2014;9:2196-5226.
24. Mishra PP, Prakash V. Mucoid and Nonmucoid Pseudomonas aeruginosa Isolated from Respiratory Tract Infections. International Journal of Advanced & Integrated Medical Sciences 2016;1:43-45.
25. Srifuengfung S, Tiensasitorn C, Yungyuen T, Dhiraputra C. Prevalence and antimicrobial susceptibility of P.aeruginosa mucoid and non-mucoid type. Southeast Asian J Trop Med Public Health 2004;35:893-896.
26. Shawar RM, MacLeod DL, Garber RL, Burns JL, Stapp JR, Clausen CR, Tanaka SK. Activities of tobramycin and six other antibiotics against P. aeruginosa isolates from patients with cystic fibrosis. Antimicrob Agents Chemother 1999;43:2877-2880.
27. Ciofu O, Fussing V, Bagge N, Koch C, Hoiby N. Characterization of paired mucoid/non-mucoid P. aeruginosa isolates from Danish cystic fibrosis patients: antibiotic resistance, β-lactamase activity and riboprinting. J Antimicrob Chemother 2001;48:391-396.
28. Codjoe FS and Donkor ES. Carbapenem Resistance: A Review. Med. Sci. 2018;6:1.
29. 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. Indian J Med Microbiol 2008;26:233-7.
30. Murugan S, Lakshmi RB, Uma DP, Mani KR. Prevalence and antimicrobial susceptibility pattern of metallo beta lactamase producing Pseudomonas aeruginosa in diabetic foot infection. Int J Microbiol Res 2010;1:123-8.
31. Varaiya A, Kulkarni N, Kulkarni M, Bhalekar P, Dogra J. Incidence of metallo beta lactamase producing Pseudomonas aeruginosa in ICU patients. Indian J Med Res 2008;127:398-402.
32. Franklin C, Liolios L, Peleg AY. Phenotypic detection of carbapenem-susceptible metallo-beta-lactamase-producing gram-negative bacilli in the clinical laboratory. J Clin Microbiol 2006;44:3139-44.
33. Sinha S, Singh A, Verma RK, Singh DP et al. Prevalence of Metallo-betalactamases producing Pseudomonas aeruginosa in hospitalized patients in rural tertiary care hospital in Uttar Pradesh, India. Int J Med Sci. 2018;6:3099-3104.
34. Siti Nur Atiquah Idris et al. Antimicrobial susceptibility pattern and distribution of EXO U and EXO S in clinical isolates of pseudomonas aeruginosa at a Malaysian hospital.
35. S. Paranjothi and R. Dheepa; screening for multidrug resistance bacteria Pseudomonas aeruginosa in hospitalized patients in Hosur, Krishnagiri (dt),

International Journal of Pharma and Biosciences. Vol.1/ Issue-3/2010.

36. Senthamarai S, Reddy SK, Sivasankari S, Anitha C et al. Resistance pattern of Pseudomonas aeruginosa in a tertiary care hospital of Kanchipuram, Tamilnadu, India. Journal of Clinical and Diagnostic Research. 2014;8:C30-DC32.
37. Biswal I, Arora BS, Kasana D, Neetushree. Incidence of Multidrug Resistant Pseudomonas aeruginosa Isolated from Burn patients and Environment of Teaching Institution. Journal of clinical and diagnostic research. 2014;8:DC26-DC29.
38. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012; 18:268-81.
39. Noyal MJC, Menezes GA, Harish BN, Sujatha S et al. Simple screening tests for detection of carbapenems in clinical isolates of gram-negative bacteria. Indian J Med Res 2009;129:707-712.
40. Attal RO, Basak S, Mallick SK, Bose S et al. Metallo-beta-lactamase Producing Pseudomonas Aeruginosa: An Emerging Threat To Clinicians. Journal of Clinical and Diagnostic Research. 2010;4:2691-2696.

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