IDENTIFICATION OF SERINE CARBAPENEMASE AND METALLOCARBAPENEMASE ENZYMES IN PSEUDOMONAS AERUGINOSA IN GEMS MEDICAL COLLEGE, RAGOLU, SRIKAKULAM

Radhika Budumuru[1], Sampathirao Jyothi Prakash Raju[2]

[1]Assistant Professor, Department of Microbiology, GEMS Medical College& Hospital, Ragolu, Srikakulam.
[2]Assistant Professor, Department of Paediatrics, GEMS Medical College& Hospital, Ragolu, Srikakulam.

ABSTRACT

Various carbapenems have been reported in Pseudomonas aeruginosa such as VIM, NDM & OXA-48, etc. In addition, carbapenemase producers are usually associated with many other non–β-lactam resistance determinants which give rise to multidrug and pan drug resistant isolates. Detection of these enzymes in infected patients and in carriers are the two main approaches for prevention of their spread. Potential carbapenemase producers are currently screened first by susceptibility testing, using breakpoint values for carbapenems. However, many carbapenemase producers do not confer obvious resistance levels to carbapenems. So there is need for Laboratories to search for carbapenemase producers. In such instance, phenotypic based test such as Modified Hodge Test (MHT) is very much useful in confirming in vitro production of carbapenemase enzymes. But this test does not differentiate serine carbapenemase enzyme (i.e. Ambler class A & C) from metallocarbapenemase (i.e. Ambler class B). To differentiate these two enzymes, MHT positive isolates can be subjected to Disc Synergy test. These two tests are highly sensitive and specific and adaptable to any laboratory in the world. Out of 100 ceftazidime resistant Pseudomonas aeruginosa, 75(75%) were sensitive, 7(7%) were intermediate sensitive and 18(18%) were resistant to imipenem. When the 18 imipenem resistant strains were subjected to Modified Hodge test, 15 gave positive results. When the 15 MHT positive strains subjected to disc synergy test, 8 were positive and 7 were negative showing that 8 were producing metallocarbapenemases and 7 were producing serine carbapenemases. Out of 7 intermediately imipenem sensitive isolates, 2 were producing metallocarbapenemase and 3 were producing serine carbapenemase. Hence, total number of imipenem resistant Pseudomonas aeruginosa isolates were 23.

KEYWORDS
Imipenem, Serine Carbapenemase, Metallocarbapenemase, Modified Hodge Test, Disc Synergy Test.

HOW TO CITE THIS ARTICLE: Budumuru R, Raju SJP. Identification of serine carbapenemase and metallocarbapenemase enzymes in Pseudomonas aeruginosa in GEMS Medical College, Ragolu, Srikakulam. J. Evid. Based Med. Healthc. 2016; 3(44), 2201-2207. DOI: 10.18410/jebmh/2016/488

INTRODUCTION: Carbapenemases increasingly have been reported in Pseudomonas spp in the past 10 years. Metallo-enzymes (VIMs, IMP) also have been reported worldwide, with a higher prevalence in southern Europe and Asia. Carbapenemases of the Oxacillinase-48 type have been identified mostly in Mediterranean and European countries and in India. Recent identification of New Delhi metallo-β-lactamase-1 producers, originally in the United Kingdom, India, and Pakistan and now worldwide, is worrisome.

Detection of infected patients and carriers with carbapenemase producers is necessary for prevention of their spread. Identification of the carbapenemase genes relies mostly on molecular techniques, whereas detection of carriers is possible by using screening culture media. This strategy may help prevent development of nosocomial outbreaks caused by carbapenemase producers, particularly Pseudomonas spp. Resistance may be related to association of a decrease in bacterial outer-membrane permeability, with overexpression of β-lactamases with carbapenemase activity.

Financial or Other, Competing Interest: None.
Submission 18-05-2016, Peer Review 20-05-2016,
Acceptance 24-05-2016, Published 02-06-2016.
Corresponding Author:
Dr. Radhika Budumuru,
Plot No. 485, 9th Lane, P. N. Colony,
Srikakulam-532005, Andhra Pradesh.
E-mail: sjyothiprakashraju@yahoo.co.in
DOI: 10.18410/jebmh/2016/488

Spread of carbapenemase producers is a relevant clinical issue because carbapenemases confer resistance to most β-lactams.

Various Carbapenemases have Been Reported In Pseudomonas Spp Such as Following Types: Pseudomonas aeruginosa carbapenemase (KPC) etc. (Ambler class-A); Verona integron-encoded metallo-β-lactamase (VIM), New Delhi metallo-β-lactamase (NDM) etc. (All Ambler class-B); and Oxacillinase-48 (OXA-48; Ambler class-D). In addition, carbapenemase producers are usually associated with many other non –β- lactam resistance determinants, which give rise to Multidrug and pan drug resistant isolates. Their current extensive spread worldwide in Pseudomonas spp is an important source of concern, as these carbapenemase producers are multidrug resistant. Detection of infected patients and of carriers are the two main approaches for prevention of their spread. +

Phenotypic and molecular-based techniques are able to identify these carbapenemase producers, although with variable efficiencies. The detection of carriers still relies mostly on the use of screening culture media. Potential carbapenemase producers are currently screened first by susceptibility testing, using breakpoint values for carbapenems. However, many carbapenemase producers do not confer obvious resistance levels to carbapenems.

So there is a need for laboratories to search for carbapenemase producers. In such instance, phenotype-based test such as the Modified Hodge test is very much useful in confirming in vitro production of carbapenemase enzyme. But this test does not differentiate serine carbapenemases i.e. Ambler class-A enzymes from metallocarbapenemases i.e. Ambler class-B enzymes. To differentiate these two enzymes, Modified Hodge test positive isolates can be subjected to Disc potentiation test. These two tests are highly sensitive and specific and adaptable to any laboratory in the world.

MATERIALS AND METHODS: A total number of 180 clinical samples were bacteriologically investigated in the present study. The material for present study was collected from patients admitted in the Department of Surgery, Septic Ward, Gynaecology, Medicine, Paediatrics, Orthopaedics, Nephrology of GEMS Medical College, Ragolu, Srikakulam, during the period from OCT. 2013 to AUG. 2014.

Inclusion Criteria: Cephalosporin resistant Pseudomonas aeruginosa isolated from various clinical samples such as Sputum, Bronchial washings, Pus, Urine, Blood.

Exclusion Criteria: Pseudomonas spp from faeces.

Sample Collection: A total number of 150 Pseudomonas aeruginosa were isolated from different samples such as Sputum, Bronchial washings, Urine, Pus and Blood but only 100 ceftazidime resistant Pseudomonas aeruginosa were included in present study.

Pus Sample: Pus sample was taken from the wound after cleaning with gauge soaked in saline. Sample was collected by swabbing the wound with two sterile swabs. One swab used for direct smear and another swab for inoculating on the solid and liquid media.

Vaginal and Cervical Swabs:

- Endocervical- Remove cervical mucus with swab and discard. Insert a second swab into endocervical canal and rotate against walls. Allow time for organisms to absorb onto the swab surface.
- Urethral- Collect urethral specimens at least 1 hr. after patient has urinated. Insert small swab 2-4 cm into urethral lumen, rotate, leave for 2s to facilitate absorption. Transport to the laboratory immediately.

Urine Sample: The patient was instructed to collect clean catch mid-stream urine in a sterile container.

Sputum Sample: The patient was instructed to collect early morning deep coughed out sputum sample in a disposable wide mouthed screw capped plastic container.

Bronchial Secretions: These were collected by using a sterile fibre optic bronchoscope.

Blood Sample: Blood was aseptically collected, 5 mL from children and 10 mL for adults. The skin overlying the vein was cleaned with soap and water. After that the area cleaned with 70% alcohol and finally painted with povidone-iodine in alcohol. Once the area was dry, the specimen was collected using a perfectly dry, sterile syringe and needle. The needle was then withdrawn and removed from the syringe prior to inoculation of the sample in to the bottle. The specimen was preferably collected in Hartley's broth at the onset of fever and sub cultured on Nutrient Agar, Blood Agar and MacConkey's Agar and incubated aerobically at 37^0C for 18 hours and then examined. All non-lactose fermenting on colonies from MacConkey's medium resembling Pseudomonas aeruginosa and green coloured colonies seen on nutrient agar were subjected to a battery of tests as follows:

1. Selective cetrimide media can be used for enhanced isolation.
2. Gram-staining for morphology.
3. Hanging drop for motility.
4. For production of enzymes- Oxidase, Catalase, Nitrate reduction, Urease.
5. For substrate utilisation – A) Citrate utilisation test, B) malonate utilisation test.
6. For metabolism of proteins and aminoacids-indole production.
7. Tests for specific breakdown products- Methyl red test, Voges-Proskauer test (Acetoin production).
8. Tests for utilisation of carbohydrates of sugar media containing Glucose, Lactose, Xylose, Sucrose, Maltose, Mannitol.
9. Antibiotic sensitivity testing by Kirby-Bauer disc diffusion method for the drug ceftazidime was done first. (Only ceftazidime resistant Pseudomonas aeruginosa strains were included in the present study).
10. Antibiotic sensitivity testing for the drug Imipenem to ceftazidime resistant pseudomonas aeruginosa by Kirby- Bauer disc diffusion method.
11. Imipenem resistant and intermediate sensitive strains were subjected to Modified Hodge test.
12. Modified Hodge test positive strains were subjected to Disc synergy test.

Method of Modified Hodge Test (MHT) (Recommended by CLSI 2010):0.5 McFarland standard suspension of E. coli ATCC 25922(indicator strain) was prepared in a broth and diluted to 1:10 in broth. Indicator strain (E. coli ATCC 25922) was streaked as a lawn on Mueller Hinton agar plate. An imipenem disk was placed in the middle of the agar plate after background lawning. 3-4 colonies of the test isolate were taken with a sterile loop and streaked on the plate from imipenem disk to periphery. Carbapenemase producing isolate was detected by the MHT when the test isolate produces the enzyme and allows growth of a carbapenem susceptible strain (Indicator strain E. coli ATCC 25922) towards an imipenem disk.

The result is a characteristic cloverleaf-like indentation in case of carbapenemase production.

The plates are examined after 16–24 hours of incubation for a cloverleaf type indentation at the intersection of the test organism and the E. coli 25922 within the zone of inhibition of the imipenem disc.

Interpretations of the Diameters of Zone of Inhibition are as follows:

MHT Positive: Test has a cloverleaf-like indentation of the E. coli 25922 growing along the test organism growth streak within the disk diffusion zone indicating that this isolate is producing a carbapenemase.

MHT Negative test has no growth of the E. coli 25922 along the test organism growth streak within the disc diffusion indicating that this isolate is not producing a carbapenemase.

Quality Control Testing:

1. Positive control (MHT positive Pseudomonas aeruginosa-27853 BD Phoenix strain) and negative control (Pseudomonas aeruginosa- In house control strain)
2. To differentiate serine carbapenemase from metallocarbapenemase, MHT positive strains are subjected to disc synergy test using imipenem (10 micrograms) and imipenem – EDTA combination discs (10 micrograms) (Himedia labs) [Patricia Marchiaro et al].

METHOD OF DISC SYNERGY TEST (DST) (Spyros Pournaras Et Al 2010, Kenneth S. Thomson 2010, Patricia Marchiaro Et Al): Mueller Hinton agar was inoculated with an overnight culture of test strain, previously adjusted to 0.5 McFarland standard turbidity, using broth or saline according to CLSI recommendations. Imipenem and imipenem-EDTA discs were placed at a distance of 10 mm from one another in the center of the plate. The plates were examined after 18-24 hrs. of incubation at 37°c. The test is said to be positive when the zone of inhibition around the imipenem with EDTA disc is >5 mm compared to zone of inhibition around imipenem disc.

RESULTS AND DISCUSSION: A total of 100 third generation cephalosporin resistant Pseudomonas aeruginosa, were isolated from different samples.

Age (Years)	No. of. Isolates	% of isolates
1-10	10	10%
11-20	14	14%
21-30	2	21%
31-40	13	13%
41-50	22	22%
1-60	12	12%
61& Above	8	8%
Total	**100**	**100%**

Table 1: Ceftazidime resistant Pseudomonas aeruginosa Isolates-Age-wise Distribution (n=100)

Pseudomonas aeruginosa isolates showed higher prevalence in 41-50 yrs. age group (22%) followed by 21-30 yr.

Age group (21%). 10%, 14%, 13%, 12%, 8% Pseudomonas aeruginosa were isolated in 1-10 yrs., 11-20 yrs., 31-40 yrs., 51-60 yrs. and 61 yrs. & above age groups respectively.

Sl. No.	Sample	No. of Isolates	Percentage
1	Sputum	45	45%
2	Pus	21	21%
3	Urine	20	20%
4	Vaginal and cervical	7	7%
5	Bronchial washings	4	4%
6	Blood	3	3%
	Total	**100**	**100%**

Table 2: Distribution of Pseudomonas aeruginosa from Different Samples were as Follows (n=100)

Of the 100 isolates, 45(45%) were isolated from sputum samples, 21(21%) were from pus samples, 20(20%) were from urine samples, 7(7%) were vaginal and cervical swabs, 4(4%) were bronchial washings and 3(3%) were blood samples.

Imipenem (10 µg)	No. of Samples	Percentage
Sensitive	75	75%
Intermediate sensitive	7	7%
Resistant	18	18%

Table 3: Antibiotic Sensitivity Pattern to Imipenem (n=100)

Out of 100 Pseudomonas aeruginosa isolates, 75(75%) were imipenem sensitive, 7(7%) were intermediately sensitive to imipenem and 18 were imipenem resistant which accounts for 18% of total samples.

Specimens	Sensitive	Intermediately Sensitive	Resistance
Sputum (n=45)	35 (77.77%)	3(6.66%)	7(15.55%)
Pus (n=21)	17(80.95%)	1(4.76%)	3(14.28%)
Urine (n=20)	16(80%)	-	4(20%)
Vaginal swabs and cervical swabs (n=7)	3(42.85%)	3(42.85%)	1(14.28%)
Bronchial washings (n=4)	2(50%)	-	2(50%)
Blood (n=3)	2(66.66%)	-	1(33.33%)
Total	**75**	**7**	**18**

Table 4: Sample wise Antibiotic Sensitivity Pattern to Imipenem

Out of 45 Pseudomonas aeruginosa isolates from sputum, 77.77% were imipenem sensitive, 6.66% were intermediately sensitive to imipenem and 15.55% were resistant imipenem. Out of 21 Pseudomonas aeruginosa isolates from pus, 80.95% were imipenem sensitive, 4.76%

were intermediately sensitive to imipenem and 14.28% were resistant to imipenem.

Out of 7 Pseudomonas aeruginosa isolates from vaginal and cervical swabs, 42.85% were imipenem sensitive, 42.85% were intermediately sensitive to imipenem and 14.28% were resistant to imipenem. Out of 4 Pseudomonas aeruginosa isolates from bronchial washings, 50% were imipenem sensitive, and 50% were resistant imipenem. Out of 4 Pseudomonas aeruginosa isolates from urine, 80% were imipenem sensitive, and 20% were resistant to imipenem. Out of 4 Pseudomonas aeruginosa isolates from urine, 66.66% were imipenem sensitive, and 33.33% were resistant to imipenem. When imipenem resistant strains were subjected to Modified Hodge test (MHT) the results were as shown in Table-5.

MHT	Number	Percentage
Positive	15	83.33%
Negative	3	16.67%

Table 5: Modified Hodge test for Imipenem Resistant Strains (n=18)

Of the 18 strains that ware imipenem resistant, 15 were found to be carbapenemase producers by Modified Hodge test. When intermediately sensitive imipenem strains were subjected to modified Hodge test the results were as shown in Table-6.

MHT	Number	Percentage
Positive	5	71.42%
Negative	2	28.57%

Table 6: Modified Hodge Test for Intermediately Sensitive Imipenem Strains (n=7)

Of the 7 that are intermediately imipenem sensitive strains, 5 were found to be carbapenemase producers by Modified Hodge test.

MHT Positive	Disc Synergy Test Positive	Disc Synergy Test Negative
15	8	7

Table 7: Disc Synergy Test for Imipenem Resistant Strains (n=15)

When the MHT positive imipenem resistant strains were subjected to disc synergy test the results were as shown in Table-7. Out of 15 MHT positive imipenem resistant isolates, 8 were positive and 7 were negative for disc synergy test indicating that 8 were producing metallocarbapenemase and 7 were producing serine carbapenemase.

MHT Positive	Disc Synergy Test Positive	Disc Synergy Test Negative
5	2	3

Table 8: Disc Synergy Test for Intermediately Sensitive Imipenem Strains (n=5)

When the MHT positive intermediately sensitive imipenem strains were subjected to disc synergy test, the results were as follows (n=5). Out of 5 MHT positive, intermediately imipenem sensitive isolates 2 were positive and 3 were negative for Disc synergy test indicating that 2 were producing metallocarbapenemase and 3 were producing serine carbapenemase.

Samples	No. of Serine Carbapenemase Producers and %	No. of Metallocarbapenemase Producers and %
Sputum (n=45)	5(11.11%)	3(6.66%)
Urine (n=20)	2(10%)	1(5%)
Pus (n=21)	1(4.76%)	2(9.52%)
Vaginal and cervical swabs(n=7)	1(14.28%)	2(28.57%)
Blood (n=3)	1(33.33%)	0
Bronchial washings(n=4)	0	2(50%)

Table 9: Distribution of Serine and Metallo-carbapenemase Enzyme Production in Pseudomonas Aeruginosa Isolates from Different Samples

Out of 45 Pseudomonas aeruginosa isolates from sputum samples, 11.11% produced serine carbapenemase and 6.66% produced metallocarbapenemase. Out of 20 Pseudomonas aeruginosa isolates from urine samples 10% produced serine carbapenemase and 5% produced metallocarbapenemase. Out of 21 Pseudomonas aeruginosa isolates from pus samples 4.76% produced serine carbapenemase and 9.52% produced metallocarbapenemase. Out of 7 Pseudomonas aeruginosa isolates from vaginal and cervical swabs, 14.28% produced serine carbapenemase and 28.57% produced metallocarbapenemase. Out of 3 Pseudomonas aeruginosa isolates from blood samples, 33.33% produced serine carbapenemase and none of them produced metallocarbapenemase. Out of 4 Pseudomonas aeruginosa isolates from bronchial washings, 50% produced metallocarbapenemase and none of them produced serine carbapenemase.

DISCUSSION: The present study was conducted to isolate and identify carbapenemase producing isolates.

Author	No. of Imipenem Resistant Isolates	No. of Isolates Positive for MHT	No. of Isolates Positive for DST	% of Serine Carbapenemase	% of Metallocarbapenemase
P Jemima et al[1]	57	57	57	-	100%
Present Study	23	20	10	43.48%	43.48%
Sundararaj Jeremiah et al[2]	100	93	86	7%	86%
Saurav Jyothi Pragathi et al	550	22	Not Done	4.7% were carbapenemase producers	
Fareya Haider et al[2]	12	9	8	4.1%	66.4%
Joan Ascnath Chelakumari et al[2]	20	9.	Not Done	11% were carbapenemase producers	

Table 10: Comparison of Positivity of Modified Hodge Test and Disc Synergy Test and % of Serine and Metallocarbapenemase Production with Indian Studies

Different Studies	No. of. Isolates	No. of. Imipenem Resistant Isolates	% of Imipenem Resistance
P Giakkoupi et al[3]	17	14	82.35%
P Jemima et al	100	57	57%
K F Anderson et al	96	42	43.75%
Patricia et al	13	3	23.07%
Mohammed Akram et al[4]	22	3	12%
Kyungwon Lee et al	-	-	6%
Ekta Gupta et al[5]	343	15	4.37%
I Shukla et al	120	0	0%
Present study	100	23	23%

Table 11: Comparison of Resistant Pattern of Imipenem in Different Studies

Pseudomonas aeruginosa from sputum samples was high with a significant p value of <0.0001. Out of the 100 ceftazidime resistant Pseudomonas aeruginosa isolates, 75 were imipenem sensitive, 7 were intermediately sensitive to imipenem and 18 were imipenem resistant accounting for 75%, 7% and 18 % respectively. This shows that there is resistance to carbapenems to a considerable degree with a significant value of <0.005. When the 18 imipenem resistant strains were subjected to Modified Hodge test, 15 gave positive result. This indicates that these isolates are producing carbapenemase enzymes.

To distinguish whether the produced carbapenemase is serine or metallocarbapenemase these 15 MHT positive strains were subjected to disc synergy test, 8 were positive and 7 gave negative results, showing that 8 were producing metallocarbapenemase and 7 were producing serine carbapenemase (Table-11). Remaining 3 imipenem resistant strains did not produce any of these carbapenemases but they are resistant because resistance to carbapenems is multimodal, one of them being enzyme production. As the presence of carbapenemase does not always result in high level resistant to carbapenems and it may also cause zone of inhibition to remain within intermediate range, the intermediately imipenem sensitive isolates were also subjected to MHT.

Author	No. of Imipenem Resistant Isolates	No of Isolates Positive for MHT	No. of Isolates Positive for DST	% of Serine Carbapenemase	% of metallocarbapenemase
K. F. Anderson et al[6] (Atlanta,2007)	42	42	0	100%	-
G. Meletis et al(2010)	570	Not Done	Not Done	47%	53%
Patrice Nordmann et al (Paris,2012)[7]	88	Not Done	Not Done	35.22%	45.45%

P Giakkoupi et al (Greece,2003)	14	14	14	-	100%
Present Study	23	20	10	43.48%	43.48%

Table 12: Comparison of Positivity of Modified Hodge Test and Disc Synergy Test and % of Serine and Metallocarbapenemase Production in Different Studies with Foreign Studies

Out of 7 intermediately imipenem sensitive isolates, 5 were MHT positive indicating that they were producing carbapenemases and were resistant to imipenem. When these 5 MHT positive strains were subjected to Disc synergy test, 2 were positive and 3 were negative.

Hence 2 were producing metallocarbapenemase and 3 were producing serine carbapenemase. Thus, the remaining 2 MHT negative isolates were sensitive to imipenem. The percentage of Imipenem resistance in present study was nearly equal to the imipenem resistance pattern of Patrica et al and Mohammed Akram et al. The percentage of imipenem resistance of P. Giakkoupi et al, K.F. Anderson et al and P. Jamima et al are higher than that of present study. The percentage of imipenem resistance of I. Shukla et al, Kyungwon Lee et al, Ekta Gupta et al are less than that of present study.

In the present study, 23 Pseudomonas aeruginosa were imipenem resistant, of which 20 were MHT positive and 10 were DST positive indicating 43.48% were producing serine carbapenemase and 43.48% producing metallocarbapenemase. In remaining 14% of isolates, the resistance mechanism was not identified. P Giakkoupi et al reported that 100% of imipenem resistant Pseudomonas aeruginosa were producing metallocarbapenemase. K. F Anderson et al reported 100% of imipenem resistant Pseudomonas aeruginosa are producing serine carbapenemase. G. Meletis et al reported that 47% were producing serine carbapenemase and 53% were producing serine carbapenemase.

Patrice Nordmann et al reported that 35.22% were producing serine carbapenemase and 45.45% were producing serine carbapenemase. In both the above studies, serine and metallocarbapenemase production was detected by PCR. The results of present study was nearly equal to that of G.Meletis et al and Patrice Nordmann et al studies.

In the present study 23 Pseudomonas aeruginosa were imipenem resistant, of which 20 were MHT positive and 10 were DST positive indicating 43.48% were producing serine carbapenemase and 43.48% producing metallocarbapenemase. In remaining 14% of isolates, the resistance mechanism was not identified. P. Jamima et al have reported that all the 57 imipenem resistant Pseudomonas aeruginosa isolates were positive for MHT and DST indicating that 100% were producing metallocarbapenemase. Saurav Jyothi Pragathi et al and Joan Ascnath Chelakumari et al reported that 4.7% and 11% of imipenem resistant Pseudomonas aeruginosa were producing carbapenemase enzymes respectively by modified Hodge test only.

Sundararaj Jeremiah et al reported out of 100 imipenem resistant Pseudomonas aeruginosa 93 were MHT positive and 86 were DST positive. That means 7% were producing serine carbapenemases and 86% were producing metallocarbapenemases. The resistant mechanism for remaining 7% of imipenem resistant isolates was not identified. Fareya Haider et al reported out of 12 imipenem resistant Pseudomonas aeruginosa, 9 were MHT positive and 8 were DST positive. That means 4.1% were producing serine carbapenemases and 66.4% were producing metallocarbapenemases. The resistant mechanisms for remaining 29.5% imipenem resistant isolates were not identified.

Confirmation of Carbapenemase Producing Pseudomonas Aeruginosa by Modified Hodge Test is a Crucial Infection Control Issue Because:

1) More resistant organisms such as Pseudomonas aeruginosa that has acquired a carbapenemase can act as a vector responsible for carbapenemase transmission to other members of the family Enterobacteriaceae in which resistance mechanism is not recognised.
2) Those isolates that are expressing these enzymes are characterised by reduced susceptibility to imipenem, but zone of inhibition ranges from sensitive to fully resistant, so resistance to these isolates may go unnoticed following routine susceptibility testing.

It was recognised that MHT is not specific for the type of carbapenemase and may give positive results with any enzyme with carbapenemase activity thus requiring PCR for the differentiation of the carbapenemase present. But so many labs do not have the facility to perform PCR. In this regard there is increasing interest in the use of EDTA compounds that seems to be promising candidate for the detection of potent metallocarbapenemase. Phenotypic tests based on the inhibitory activity of EDTA-a chelating agent are very easy to perform, interpret and reproducible.

Distinguishing serine carbapenemase from metallobetalactamase by disc synergy test helps to direct treatment and emphasizes that those isolates that are producing serine carbapenemases can be inhibited by clavulanic acid and tazobactam and those isolates that are producing metallocarbapenemases are inhibited by EDTA, a chelator of Zn+ and other divalent cations and resist currently available β-lactamase inhibiters such as clavulanic acid and tazobactam and lack the ability to hydrolyse aztreonam. The drugs used for treating metallocarbapenemase producing isolates are Tigecycline, Colistin, Polymyxin-B. The percentage of serine & metallocarbapenemase enzyme production by K. P. Pneumoniae of Patrice Nordmann et al coincides with that of present study (Table-12).

The percentage of serine carbapenemase enzyme production by K. P. Ozaenae of Patrice Nordmann et al was more than that of present study. The percentage of serine and metallocarbapenemase enzyme production by Pseudomonas aeruginosa strains isolated from bronchial washings of Payal Desh et al[8] study was equal to that of present study (Table-12). The percentage of serine and metallocarbapenemase enzyme production by Pseudomonas aeruginosa strains isolated from sputum samples of Payal Desh et al study coincides with that of present study.

The percentage of serine carbapenemase enzyme production by Pseudomonas aeruginosa strains isolated from urine, swabs, blood, pus samples of Payal Desh et al[8] study was less than of present study.

The percentage of metallocarbapenemase enzyme production by Pseudomonas aeruginosa strains isolated from blood samples of Payal Desh et al study was more than of present study. While carbapenem resistance in Pseudomonas and Acinetobacter spp is well known, resistance among Enterobacteriaceae is increasing now a days especially in Pseudomonas aeruginosa. Pseudomonas aeruginosa is recognized as an important reservoir for a variety of resistance determinants.

Two major types of acquired carbapenemases have been reported in Pseudomonas aeruginosa, the molecular class B metallo-β- lactamases (MBLs) and the molecular class A serine carbapenemases. Serine carbapenemases were initially restricted in the N.Y. City area (G. Meletis et al) and Atlanta area (K. F. Anderson et al), then these enzymes have been detected in countries outside the USA and recently in Europe where they have been associated with large outbreaks (Patrice Nordmann et al). In contrast, MBLs have been reported throughout the world. The emergence of a clinical Pseudomonas aeruginosa isolate possessing two different carbapenemases, serine and metallo- β-lactamases is of great concern. The present study emphasises on the isolation of a carbapenem resistant Pseudomonas aeruginosa isolates producing both serine and carbapenemases.

Recently, three strains of Pseudomonas aeruginosa co-producing both carbapenemases have been isolated from clinical specimens in Greek Hospitals (P.Giakkoupi et al 2009 and D. Radhakrishna, 2015).[9] Simultaneous production of both enzymes by Pseudomonas aeruginosa isolates was also observed in other studies. (G.Meletis et al, Patrice Nordmann et al, Sundararaj Jeremiah et al, Fareya Haider et al.)

These findings indicate the continued spread of resistance genes among these pathogens. The concomitant presence of both enzymes poses clinical and therapeutic problems. Both serine and metallocarbapenemase enzymes reside on mobile genetic elements and are transferable. Furthermore, apart from the broad hydrolysis activity of carbapenemases, most of the isolates possess other mechanisms of resistance, leaving limited options for antimicrobial regimens. Therefore, it is essential to control their spread to other bacterial species or to unrelated clones. The detection of serine and metallocarbapenemase coproducing isolates is difficult and requires the use of reliable confirmatory methods. It is of clinical importance that laboratories adopt a simple and reliable phenotypic screening test to identify promptly and accurately these organisms for both therapeutic considerations and infection control purposes.

REFERENCES

1. Hesna Yigit, Anne Marie Queenan, Gregory J Anderson, et al. Novel carbapenem-hydrolysing β-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumonia. Antimicrob Agents Chemother 2001;45(4):1151-1161.
2. Radhika B, Jyothipadmaja I. Subspeciation and antibacterial susceptibility testing of Klebsiella pneumonia. J Pub Health Med Res 2014;2(2):39-42.
3. Giakkoupi P, Xanthaki A, Kanelopoulou M, et al. VIM-1 metallo-β-lactamase producing pseudomonas aeruginosa strains in Greek hospitals. J Clin Microbiol 2003;41(8):3893-3896.
4. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. Annals of Clinical Microbiology and Antimicrobials 2007;6(4):1-7.
5. Ekta Gupta, Srujana Mohanty, Seema Sood, et al. Emerging resistance to carbapenems in a tertiary care hospital in north India. Indian J Med Res 2006;124(1):95-98.
6. Anderson KF, Lonsway DR, Rasheed JK, et al. Evaluation of methods to identify the Klebsiella pneumoniae carbapenemase in Enterobacteriaceae. J Clin Microbiol 2007;45(8):2723-2725.
7. Patrice Nordmann, Laurent Poirel, Laurent Dortet. Rapid detection of carbapenemase producing Enterobacteriaceae: emerging infectious diseases. 2012;18(9):1503-1507.
8. Payal Deshpande, Camilla Rodrigues, Anjali Shetty, et al. New Delhi metallo-β lactamase (NDM-1) in Enterobacteriaceae: treatment options with carbapenems compromised. JAPI 2010;58:147-149.
9. Radha Krishnan D, Barama Srihari. A study on the severity of right ventricular dysfunction in correlation with the severity of lung dysfunction in chronic obstructive pulmonary disease patients-COP. The Am J Sci & Med Res 2015;1(1):112-119.