



Detection of serinecarbapenemase and Metallo carbapenemase enzymes in *Klebsiella pneumoniae* in a tertiary care hospital

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Abstract

Various carbapenems have been reported in *K.pneumoniae* such as KPC, 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 1st 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 invitro production of carbapenemase enzymes. But this test does not differentiate serine carbapenemase enzyme (i.e. Ambler class A & C) from metallo carbapenemase (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 *K.pneumoniae* 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 metallo carbapenemases and 7 were producing serine carbapenemases. Out of 7 intermediately imipenem sensitive isolates 2 were producing metallo carbapenemase and 3 were producing serine carbapenemase. Hence total number of imipenem resistant *K.pneumoniae* isolates were 23.

Keywords: Imipenem, Serine carbapenemase, Metallo carbapenemase, Modified Hodge test, Disc Synergy test.

INTRODUCTION

Carbapenemases increasingly have been reported in *Enterobacteriaceae* in the past 10 years. *Klebsiella pneumoniae* carbapenemases have been reported in the United States and then worldwide, with a marked endemicity at least in the United States and Greece. 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

How to Cite this Article:

B. Radhika and I. Jyothi Padmaja (2015). Detection of serinecarbapenemase and Metallo carbapenemase enzymes in *Klebsiella pneumoniae* in a tertiary care hospital. *The Ame J Sci & Med Res*, 1(1):136-147.
doi:10.17812/ajsmr20151123.
Published online 3 August, 2015

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 *K. pneumonia* [27].

Resistance may be related to association of a decrease in bacterial outer-membrane permeability, with overexpression of β -lactamases with carbapenemase activity. Spread of carbapenemase producers is a relevant clinical issue because carbapenemases confer resistance to most β -lactams [26].

Various carbapenemases have been reported in *Klebsiella pneumoniae* such as following types: *Klebsiella pneumoniae* 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) [26].

In addition, carbapenemase producers are usually associated with many other non- β -lactam resistance determinants, which give rise to multidrug and pandrug resistant isolates [26].

Their current extensive spread worldwide in *K. pneumoniae* 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 [28].

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 [26].

In such instance, phenotype-based test such as the Modified Hodge test is very much useful in confirming invitro production of carbapenemase enzyme. But this test does not differentiate serine carbapenemases i.e Ambler class-A enzymes from metallo carbapenemases 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 were collected from patients admitted in the department of Surgery, Septicward, Gynecology, Medicine, Paediatrics, Orthopaedics, Nephrology of King George Hospital, Visakhapatnam and from the patients attending Government Hospital for Chest and Communicable Diseases, Visakhapatnam during the period from OCT 2010 to AUG 2012.

- **Inclusion Criteria:** Cephalosporin resistant *Klebsiella pneumoniae* isolated from various clinical samples such as sputum, bronchial washings, pus, urine, blood.
- **Exclusion Criteria:** *Klebsiella* spp from faeces.

Sample Collection:

A total number of 150 *Klebsiella pneumoniae* were isolated from different samples such as Sputum, Bronchial washings, Urine, Pus and Blood.

Pus Sample:

Pus sample was taken from the wound after cleaning with gauze 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.

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 fiberoptic bronchoscope.

Blood Sample:

Blood was collected by strict aseptic technique. The skin overlying the vein was vigorously wiped with soap and water. After that the area was

cleaned with 70% alcohol and finally painted with povidine-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 at the onset of fever.

All the specimens were inoculated on Nutrient Agar, Blood Agar and MacConkey's Agar and incubated aerobically at 37°C for 18 hours and then examined. All lactose fermenting mucoid colonies from MacConkey's medium resembling *Klebsiella* species were subjected to a battery of tests as follows.

1. Gram staining for morphology
2. Hanging drop for motility
3. Capsular staining using congo red
4. For production of enzymes - Oxidase, Catalase, Nitrate reduction, Urease
5. For substrate utilization – A) Citrate utilization test, B) Malonate utilization 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 *Klebsiella pneumoniae* strains were included in the present study)
10. Antibiotic sensitivity testing for ceftazidime resistant strain by Kirby-Bauer disc diffusion method for the drug Imipenem.
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. A 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 a 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 clover- leaf 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 clover leaf-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:

Positive control (MHT positive *Klebsiella pneumoniae*- In house control strain) and negative control (*Klebsiella pneumoniae* MTCC 3384)

- To differentiate serine carbapenemase from metallo carbapenemase, MHT positive strains are subjected to disc synergy test using imipenem (10 micrograms) and imipenem – EDTA combination discs(10 micrograms)(Hi media 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 10mm from one another in the center of the plate. The plates were examined after 18-24hrs of incubation at 37°C. The test is said to be positive when the zone of inhibition around the

imipenem with EDTA disc is >5mm compared to zone of inhibition around imipenem disc .

RESULTS AND DISCUSSION

A total of 100 third generation cephalosporin resistant *Klebsiella pneumoniae*, were isolated from different samples.

Table-1. Ceftazidime resistant *Klebsiella pneumoniae* isolates- Age-wise distribution (n=100)

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%

Klebsiella pneumoniae isolates showed higher prevalence in 41-50yrs age group(22%) followed by 21-30yr age group(21%). 10%,14%,13%,12%,8% *Klebsiella pneumoniae* were isolated in 1-10yrs, 11-20yrs, 31-40yrs, 51-60yrs and 61yrs & above age groups respectively.

Table-2. Distribution of *K.pneumoniae* from different samples were as follows (n=100)

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

Out of 100 *K.pneumoniae* 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.

Table-4. Sample wise Antibiotic Sensitivity pattern to imipenem

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

Out of 45 *K.pneumoniae* isolates from sputum, 77.77% were imipenem sensitive, 6.66% were intermediately sensitive to imipenem and 15.55% were resistant imipenem. Out of 21 *K.pneumoniae* isolates from pus, 80.95% were imipenem sensitive, 4.76% were intermediately sensitive to imipenem and 14.28% were resistant imipenem.

Out of 7 *K.pneumoniae* isolates from vaginal and cervical swabs, 42.85% were imipenem sensitive, 42.85% were intermediately sensitive to imipenem and 14.28% were resistant imipenem. Out of 4 *K.pneumoniae* isolates from bronchial washings, 50% were imipenem sensitive, and 50% were resistant imipenem.

Out of 4 *K.pneumoniae* isolates from urine, 80% were imipenem sensitive, and 20% were resistant imipenem. Out of 4 *K.pneumoniae* isolates from urine, 66.66% were imipenem sensitive, and 33.33% were resistant imipenem.

Table-5. Modified hodge test for imipenem resistant strains (n=18)

MHT	Number	Percentage
Positive	15	83.33 %
Negative	3	16.67 %

When imipenem resistant strains were subjected to Modified Hodge test (MHT) the results were as shown in Table-5. Of the 18 strains that were 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.

Table- 6. Modified hodge test for intermediately sensitive imipenem strains (n=7)

MHT	Number	Percentage
Positive	5	71.42 %
Negative	2	28.57 %

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

Table – 7. Disc synergy test for imipenem resistant strains (n=15)

MHT positive	Disc synergy test Positive	Disc synergy test negative
15	8	7

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.

Table – 8. Disc synergy test for intermediately sensitive imipenem strains (n=5)

MHT positive	Disc synergy test positive	Disc synergy test Negative
5	2	3

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.

Table – 9. Distribution of serine and metallo-carbapenemase enzyme production in Klebsiella pneumoniae isolates from different samples.

Samples	No of serine carbapenemase producers & %	No of metallo carbapenemase producers & %
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%)

Out of 45 Klebsiella pneumoniae isolates from sputum samples 11.11% produced serine carbapenemase and 6.66% produced metallocarbapenemase. Out of 20 Klebsiella pneumoniae isolates from urine samples 10% produced serine carbapenemase and 5% produced metallocarbapenemase.

Out of 21 Klebsiella pneumoniae isolates from pus samples 4.76% produced serine carbapenemase and 9.52% produced metallocarbapenemas. Out of 7 Klebsiella pneumoniae isolates from vaginal and cervical swabs 14.28% produced serine carbapenemase and 28.57% produced metallocarbapenemase.

Out of 3 Klebsiella pneumoniae isolates from blood samples 33.33% produced serine carbapenemase and none of them produced metallocarbapenemase. Out of 4 Klebsiella pneumoniae 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

Table-10. Comparison of positivity of Modified Hodge test and disc synergy test & % of serine and metallo carbapenemase production with Indian studies

Author	No of imipenem resistant isolates	No of isolates positive for MHT	No of isolates positive for DST	% of serine carbapenemase	% of metallo carbapenemase
P Jemima et.al.	57	57	57	-	100%
Sundararaj Jeremiah et.al.	100	93	86	7%	86%
Saurav jyothi Pragathi et .al.	550	22	Not done	4.7% were carbapenemase producers	
Fareya Haideret.al.	12	9	8	4.1%	66.4%
Joan ascnath Chelakumari et.al.	20	9.	Not done	11% were carbapenemase producers	
Present study	23	20	10	43.48%	43.48%

Table-11. Comparison of resistant pattern of imipenem in different studies

Different studies	Number of isolates	Number of Imipenem resistant isolates	% of imipenem resistance
P Giakkoupi et al	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	22	3	12%
Kyungwon lee et al	-	-	6%
Ekta Gupta et al	343	15	4.37%
I Shukla et al	120	0	0%
Present study	100	23	23%

K.pneumoniae causing various infections from different clinical samples collected from GHCCD and KGH, a tertiary care hospital, during the period OCT 2010 to AUG 2012.

A total number of 150 *Klebsiella pneumoniae* were isolated, of which 100 were ceftazidime resistant. Only ceftazidime resistant *Klebsiella pneumoniae* were included in the present study.

Out of the 100 ceftazidime resistant *K.pneumoniae* isolates, 54 were from males and 46 were from females with a male female ratio of

1.2:1. Out of the 100 ceftazidime resistant *K.pneumoniae* isolates, 22 were from the age group 41-50 yrs followed by 21 in 21-30,14 in 11-20, 13 in 31-40,12 in 51-60,10 in 1-10, 8 in 61 &above year old individuals. Infection with *K.pneumoniae* was found to be more common in middle age group.

Ceftazidime resistant *K.pneumoniae* were predominantly isolated from sputum samples(45%) followed by pus samples (21%),urine samples (20%), vaginal and cervical swabs (7%), bronchial washings(4%), blood cultures (3%). Isolation of

Table-12. Comparison of percentage of production of serine and metallo carbapenemase enzymes by subspecies of *K.pneumoniae* in different studies.

Carbapenemase producing <i>Klebsiella pneumoniae</i> subspecies	Patrice Nordmann et al-Paris (2012)		Present study	
	No of serine carbapenemase	No of metallo carbapenemase	No of serine carbapenemase	No of metallo carbapenemase
<i>K. p.pneumoniae</i>	47(54.02%)	40(45.97%)	8(60%)	2(40%)
<i>K. p.ozaenae</i>	1(100%)	0(0%)	1(50%)	1(50%)
<i>K. p.aerogenes</i>	-	-	4(40%)	1(60%)
<i>K. p.rhinoscleromatis</i>	-	-	2(33.33%)	1(66.66%)
Total	88 (87-K.p.pneumoniae and 1-K.p.ozaenae)		20 (10- K.p.pneumoniae, 2-K.p.ozaenae,5- K.p.aerogenes, 3- K .p.rhinoscleromatis)	

Table-13. Comparison of percentage of serine and metallo carbapenemase enzyme production by *K.pneumoniae* strains isolated from different samples .

Samples	Payal Desh pande et al		Present study	
	No & % of serine carbapenemase	No & % of metallo carbapenemase	No & % of serine carbapenemase	No & % of metallo carbapenemase
Sputum	1(50%)	1(50%)	5(62.5%)	3(37.5%)
Urine	1(25%)	3(75%)	2(66.6%)	1(33.3%)
Pus	-	1(100%)	1(33.3%)	2(66.6%)
Blood	-	1(100%)	1(100%)	0
Bronchial washings	-	2(100%)	0	2(100%)
Swabs	-	1(100%)	1(33.3%)	2(66.6%)
Stool	-	1(100%)	0	0
No of <i>K.pneumoniae</i>	2	10(100%)	10	10

K.pneumoniae from sputum samples was high with a significant p value of <0.0001

Out of the 100 ceftazidime resistant *K.pneumoniae* 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 metallo

carbapenemase these 15 MHT positive stains were subjected to disc synergy test, 8 were positive and 7 gave negative results, showing that 8 were producing metallo carbapenemase 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 be remain with in intermediate range, the intermediately imipenem sensitive isolates were also subjected to MHT.

Table-13. Comparison of positivity of Modified Hodge test and disc synergy test & % of serine and metallo carbapenemase production in different studies with Foreign studies

Author	No of imipenem resistant isolates	No of isolates positive for MHT	No of isolates positive for DST	% of serine carbapenemase	% of metallo carbapenemase
K F Anderson et. al. (Atlanta,2007)	42	42	0	100%	-
G.Meletis et.al(2010)	570	Not done	Not done	47%	53%
Patrice Nordmann et al(paris,2012)	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%

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 metallo carbapenemase 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 ai, Ekta Gupta et al are less than that of present study.

In the present study 23 *K.pneumoniae* 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 metallo carbapenemase. In remaining 14% of isolates the resistance mechanism was not identified.

P Giakkoupi et. al. reported that 100% of imipenem resistant *K.pneumoniae* were producing metallo carbapenemase.

K F Anderson et. al. reported 100% of imipenem resistant *K.pneumoniae* 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 metallo carbapenemase 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 *K.pneumoniae* 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 metallo carbapenemase. In remaining 14% of isolates the resistance mechanism was not identified.
- P .Jamima et al have reported that all the 57 imipenem resistant *K.pneumoniae* isolates were positive for MHT and DST indicating that 100% were producing metallo carbapenemase.

- Saurav jyothi pragathi et.al and Joan ascnath chelakumari et.al reported that 4.7% and 11% of imipenem resistant *K.pneumoniae* were producing carbapenemase enzymes respectively by modified Hodge test only.
- Sundararaj jeremiah et.al. reported out of 100 imipenem resistant *K.pneumoniae* 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 *K.pneumoniae* 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 *K.pneumoniae* by Modified Hodge test is a crucial infection control issue because :

- 1) More resistant organisms such as *K.pneumoniae* 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 recognized.
- 2) Those isolates that are expressing these enzymes are characterized 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 reproduceble.

Distinguishing serine carbapenemase from metalloβ-lactamase 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 metallo carbapenemases are inhibited by EDTA, a chelator of Zn⁺ and other divalent cations and resist currently available β-lactamase inhibitors such as clavulanic acid and tazobactam and lack the ability to hydrolyze aztreonam. The drugs used for treating metallocarbapenemase producing isolates are Tigecycline, Colistin, Polymyxin-B.

The percentage of serine & metallo carbapenemase 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.ozzaenae* of Patrice Nordmann et al was more than that of present study.

The percentage of serine and metallocarbapenemase enzyme production by *K.pneumoniae* strains isolated from bronchial washings of payal Desh et al study was equal to that of present study ([Table-13](#)). The percentage of serine and metallocarbapenemase enzyme production by *K.pneumoniae* 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 *K.pneumoniae* strains isolated from urine, swabs, blood, pus samples of payal Desh et al study was less than of present study. The percentage of metallo carbapenemase enzyme production by *K.pneumoniae* 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 *K.pneumoniae*. *K. pneumoniae* is recognized as an important reservoir for a variety of resistance determinants. Two major types of acquired carbapenemases have been reported in *K.Pneumoniae*, 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 etal), 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 *K.pneumoniae* isolate possessing two different carbapenemases, serine and metallo- β -lactamases is of great concern. The present study emphasizes on the isolation of a carbapenem-resistant *K.pneumoniae* isolates producing both serine and carbapenemases. Recently, three strains of *K. pneumoniae* co-producing both carbapenemases have been isolated from clinical specimens in Greek hospitals (P.Giakkoupi et al 2009 and D. Radhakrishna, 2015). Simultaneous production of both enzymes by *K.pneumoniae* 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 metallo carbapenemase 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 metallo carbapenemase co-producing 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.

Competing interests

The authors have declared that no competing interests exist.

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