© 2015 Global Science Publishing Group USA

ISSN: 2377-6196
The American Journal of
SCIENCE AND MEDICAL RESEARCH, 2(1), 136-147

Research Article Open Access

Detection of serinecarbapenemase and Metallocarbapenemase enzymes in Klebsiella pneumoniae in a tertiary care hospital

B. Radhika and I. Jyothi Padmaja

GEMS Medical College, Ragolu, Srikakulam, Andhra Pradesh, India

E-mail: drradhikaprakash20@gmail.com

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 carbapenamase producers are currently screened 1st by susceptibility testing, using breakpoint values for carbapenems. However many carbapenemase producers donot 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 producton of carbapenemase enzymes. But this test doesnot 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 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 metallocarbapenemases and 7 were producing serinecarbapenemases. Out of 7 intermediately imipenem sensitive isolates 2 were producing metallocarbapenemase and 3 were producing serine carbapenemase. Hence total number of imipenem resistant *K.pneumoniae* isolates were 23.

Keywords: Imipenem, Serine carbapenemase, Metallocarbapenemase,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

How to Cite this Article:
B. Radhika and I. Jyothi Padmaja (2016). Detection of serinecarbapenemase and Metallocarbapenemase enzymes in Klebsiella pneumoniae in a tertiary care hospital. The American Journal of Science and Medical Research, 2(1):136-147. doi:10.17812/ajsmr2016211.

Received 2 December, 2016; Accepted 16 January, 2016; Published online 3 February, 2016

© 2015 Global Science Publishing Group USA

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 *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 isssue 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 differentiates 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 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.

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 fibreoptic bronchoscope.

Blood Sample:

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

© 2015 Global Science Publishing Group USA

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 37^0c 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 pneumonia 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 ontrol (*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 aroud 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 %

© 2015 Global Science Publishing Group USA

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

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 testpositive	Disc synergy testNegative
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 serinecarbapenemase.

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. Comparision 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 infectlons 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 producingKlebsiellapneumoniae subspecies	Patrice Nordmann et al-Paris (2012)		Present study	
	No of serine carbapenmase	No of metallo carbapenmase	No of serine carbapenmase	No of metallo carbapenemase
K. p.pneumoniae	47(54.02%)	40(45.97%)	8(60%)	2(40%)
K .p.ozaenae	1(100%)	0(0%)	1(50%)	1(50%)
K .p.aerogenes	-	-	4(40%)	1(60%)
K .p.rhinoscleromatis	-	-	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. Comparision of percentage of serine and metallocarbapenemase enzyme production by *K.pneumoniae* strains isolated from different samples .

Samples	Payal Desh pande et al		Present study	
	No & % of serine carbapenemase	No & % of metallocarbapenemase	No & % of serine carbapenemase	No & % of metallocarbapenemase
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 *K.pneumoniae*	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 imipenern 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 multimodel ,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 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 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 metallocarbapenemase. 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 metallocarbapenemase.

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 metallocarbapenemase. 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 imipenern 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 imipenern 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 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 metallo carbapenemases 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 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.ozaenae 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

© 2015 Global Science Publishing Group USA

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 emphasize 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 metallocarbapenemase 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.

References:

1. Anne Marie Queenan and Karen Bush Carbapenemase – a versatile β-lactamase Article - Antimicrobial Agents Chemotherapy. Johnson & Johnson Pharmaceutical Reasearch and Development, L.L.C Rantan, NJ 08869.(2001).Pg: 1151-1161.
2. Betty A.Forbes, Daniel F.Sahm, Alice S.Weissfeld Bailey & Scott's Diagnostic Microbiology 12th edition pg: 218-247
3. Colle J.G, Fraser A.G, Marmion B.P, Simmons A Mackie & Mc Cartney Practical medical Microbiology 14th edition pg:131-149)
4. Carla Fontana, Marco Favaro, Loredana sarmati, Silvia Natoli, Anna Altieri,Maria C Bossa, Silvia Minelli, Francesca Leonardis, Cartesio. Emergence of KPC-producing K.pneumoniae in Italy. Antimicrobial agents and Chemotherapy, August 2007.
5. David Greenwood, Richard C.B.Slack, John F.Peutherer A guide to Microbial Infections: Pathogenesis, Immunity, Laborator Diagnosis and Control. 16th edition, pg: 275-277
6. D.R.Arora Text book of Microbiology 3rd Edition. Pg no-356.
7. Dennis.S.Hansen, Hazel.M.Aucken, Titi Abiola, Rainer Podschun Recommended test panel for differentiation of Klebsiella species on the basis of a trilateral interlaboratory evaluation of 18 biochemical tests. Journal of Clinical Microbiology 2004,42(8):3665-9.
8. E kta Gupta, Srujana Mohanty, Seema Sood, Benu Dhawan, Bimal K. Das and Arti Kapil. Emerging resistance to carbapenems in a tertiary care hospital in North India. Indian journal of medical research, July(2006). Pg: 95-98.
9. Fareya Haider, Vineeta Mittal, M.E.Siddique. Evaluation of three phenotypic methods for detection of Metallo-beta- lactamases in Gram negative bacilli from hospitalized patients. Microbes, Molecules & Beyond, XXXV National conference of Indian.Association of Medical Microbiologists, Abstracts and Souvenir.
10. George A.Jacoby, Debra M.Mills and Nancy Chow Role of β- lactamases and porins in resistance to Ertapenem and other β- lactams in Klebsiella pneumoniae. Antimicrobial Agents Chemotherapy.(2004). Pg: 3203-3206.
11. G.Meletis, E.Tzampaz,E.Protonotariou, and Dsofianou Emergence of Klebsiella pneumoniae carrying blaKPC and blaVIM genes. Hippokratia quarterly medical journal. 2010 apr-jun 14(2)139-140.
12. Hesna Yigit, Anne Marie Queenan, Gregory J.Anderson, Antinio Domenech-Sanchez, James W.Biddle, Christine D.Steward, Sebastain Alberti, Karebn Bush and and Fred C.Tenover. Novel Carbapenemase hydrolyzing β-lactamase, KPC-1, from a Carbapenem-Resistant Klebsiella pneumoniae. Antimicrobial Agents Chemotherapy.(2001). Pg: 1151-1161.
13. J.Kamile Rasheed, James W.Biddle, Karen F.Anderson, Laraine Wash, Carol Chenoweth, John Perrin, Duane W.Newton, and Jean B. Patel. Detection of the klebsiella pneumoniae carbapenemase type-2 carbapenem- hydrolyzing enzyme in clinical isolates of citrobacter freundii and klebsiella oxytoca carrying a common plasmid. Anti – infective investigation section(G08), Centers for

Disease Control and Prevention,1600 Clifton Rd. NE,Atlanta,GA30333.(2008). Pg: 1-6.

14. Jeremiah Sundararaj Stanleyraj, Veeraraghavan Balaji, Shalini Anandan, Rani Diana Sahni, Peter John Victor. Characterization of the different carbapenem resistance mechanisms in clinical isolates of K.pneumoniae using phenotypic and genotypic methods. Microbes, Molecules & Beyond , XXXV National conference of Indian Association of Medical Microbiologists, Abstracts and Souvenir.
15. Joan Ascnath Chelakumari.J, Subha.S, Radha Madhavan, Gomathi. Emergence of K.pneumoniae carbapenemases- Type producing beta - lactamases – Klebsiella species in Teritiary Care Centre. Microbes, Molecules & Beyond , XXXV National conference of Indian Association of Medical Microbiologists, Abstracts and Souvenir.
16. K.F.Anderson, D.R.Lonsway, J.K.Rasheed, J.Biddle, B.Jensen, L.K.McDougal, R.B.Carey, A. Thompson, S.Stocker, B.Limbago and J.B.Patel. Evaluation of Methods to identify the Klebsiella pneumoniae Carbapenemase in Enterobacteriaceae. Journal of Clinical Microbiology(2007).pg: 2723-2725.
17. J.Kamile Rasheed, James W.Biddle, Karen F.Anderson, Laraine Wash, Carol Chenoweth, John Perrin, Duane W.Newton, and Jean B. Patel. Detection of the klebsiella pneumoniae carbapenemase type-2 carbapenem- hydrolyzing enzyme in clinical isolates of citrobacter freundii and klebsiella oxytoca carrying a common plasmid. Anti – infective investigation section(G08), Centers for Disease Control and Prevention,1600 Clifton Rd. NE,Atlanta,GA30333.(2008). Pg: 1-6.
18. Kenneth S.Thomson Extended spectrum-β-lactamase, AmpC, and Carbapenemase issues. Commentary- Creighton University School of Medicine, 2500 California plaza, Omaha, NE 68178.(2010). Pg: 1-20.
19. Laurent Poirel, Claire Heritier, Venus Tolun and Parice Nordmann. Emergence of Oxacillinase-mediated resistance to Imipenem in Klebsiella pneumoniae. American Society for Microbiology Antimicrobial Agents and Chemotherapy. Sep(2003).
20. Mohammed Akram, Mohammed shahid and Asad u Khan. Etiology and antibiotic resistance patterns of community – acquired urinary tract infections in JNMC Hospital Aligarh, India. Annals of Clinical Microbiology and Antimicrobials (2007). Pg : 1-7.
21. Patrica A. Braford, Carl Urban, Noriel Mariano, Steven J.Projan, James.J.Rahal, and Karen Bush. Imipenem resistance in Klebsiella pneumoniae is associated with the combination of ACT-1, a plasmid mediated AmpC – β- lactamase, and the loss of an outer membrane protein. Antimicrobial agents and Chemotherapy, March(1997). pg: 563-569.)
22. Payal Deshpande, Camilla Rodrigues, Anjali Shetty, Farhad Kapadia, Ashit Hedged, Rajeev New Delhi Metallo-β-lactamase (NDM-1) in Enterobacteriaceae: treatment options with carbapenemase compramised. Dept of Research, Dept of Lab Medicine, Dept of Medicine, P.D. Hinduja National Hospital & Medical Research Centre, Mumbai, India. Received:14-12-2009, Revised:18-01-12010;Accepted:19-01-2010.
23. Patricia Marchiaro, Viviana Ballerini,Tamara Spalding, Gabriela Cera, Maria A.Mussa, Jorgelina Moran-Barrio, Alejandro J.Vila and Andriana S.Limansky. A convenient microbiological assay employing cell free extracts for the rapid characterization of gram negetive carbapenemase producers. Published by Oxford University press on behalf of the British Society for Antimicrobial Chemotherapy. April(2008).
24. P.Giakkoupi, A.Xanthaki, M.Kanelopoulou, A.Vlahaki, V.Miriagou, S.Kontou, E.Papafraggas, H.Malamou-Lada, L.S.Tzouvelekis, N.J.Legakis, and A.C.Vatopoulos. VIM-1 Metallo-β-Lactamase producing Klebsiella pneumoniae strains in Greek Hospitals. Journal of Clinical Microbiology(2003).
25. Paul Schreckenberger, Washington Winn,Jr., Stephen Allen, William Janda, Elmer Koneman, Gary Procop, Gail Woods. Koneman's color Atlas and Textbook of Diagnostic Microbiology 6th edition , pg: 1443-1535
26. Patrice Nordmann, Laurent Poirel, Laurent Dortet Rapid detection of carbapenemase producing Enterobacteriaceae. Emerging Infectious Diseases. Vol 18,No 9, sep 2012.
27. Patrice Nordmann, Laurent Poirel,Thierry Naas Global spread of carbapenemase producing enterobacteriaceae Emerging Infectious Diseases.2011 october;17(10):1791-1798.
28. Patrice Nordmann, Laurent Poirel,M.Gniadkowski, C.G.Giske, N.Wooford,V.Miriagou,The european network on Carbapenemases. Identification and screening of carbapenemase producing Enterobactericeae. Clinical microbiology and infection.17 april 2012. Vol18. Issue 5, page 432-438.
29. Patrice Nordmann, Laurent Poirel, Amelie Carrer, Mark A.Toleman and Timothy R.Walsh. How to detect NDM-1 producers. Journal of clinical microbiology 2011 feb 49(2):718-721.
30. S.Peter Borriello, Patrick R.Murray & Guido Funke Topley & Wilson's Microbiology and Microbial infections 10th edition, Bacteriology, vol.2; pg:1476-1482
31. Saurav jyothi pragathi, Himadri dutta, Reema Nath, Gargi Choudhury, A.K.Borthakur. Detection of carbapenemase producing Gram negative isolates in a Teritiary Care Hospital in Assam. Microbes, Molecules & Beyond , XXXV National conference of Indian Association of Medical Microbiologists, Abstracts and Souvenir.
32. D. Radha Krishnan and Barama Srihari (2015). A study on the severity of right ventricular dysfunction in correlation with the severity of Lung dysfunction in Chronic Obstructive Pulmonary Disease patients -

COPD. The Ame J Sci & Med Res, 1(1):112-119. doi:10.17812/ajsmr20151120.

33. Shmuel Benenson, Violeta Temper, Mantan J.Cohen, Caemela Schwartz, Carlo Hidaigo-Grass and Colin Block. Imipenem disc for detection of KPC Carbapenemase-producing Enterobactericeae in clinical practice. Department of Clinical Microbiology and Infectious Diseases, Hadassah- Hebrew University Medical Center, P.O. Box 12000,
34. Simona Bratu, Mohamad Mooty, Satyen Nichani, David Landman, Carl Gullans, Barbara Pettinato, Usha Karumudi, Pooja Tolaney and John Quale. Emergence of KPC-possesssing Klebsiella pneumoniae in Brooklyn,New york : Epidemiology and Recommendations for detection. Antimicrobial Agents Chemotherapy.(2005). Pg: 3018-3020.
35. Spyros Pournaras,Aggeliki poulou,Athanassios Tsakris Inhibitor –based methods for detection of KPC carbapenemase-producing Enterobacteriaceae in clinical practice by using boronic acid compounds. Published by Oxford University Press on behalf of the British society for Antimicrobial chemotherapy.2010.pg no-1-8.
36. Thierry Naas, Patrice Nordmann, Gerard Vedel and Claire Poyart. Plasmid – Mediated Carbapenem-Hydrolyzing β-Lactamase KPC in a Klebsiella pneumoniae isolate from France. Antimicrobial Agents Chemotherapy.(2005). Pg: 4423-4424.
37. Ze-Qing Wei, Xiao-Xing Du, Yun-Song Yu, Ping Shen, Ya-Gang Chen, and Lan-Juan Li. Plasmid mediated KPC-2 in a Klebsiella pneumopniae isolate from China. Antimicrobial Agents Chemotherapy.(2007). Pg: 763-765.

*****