ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 01, pp.45444-45447, January, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

DETECTION OF CARBAPENEMASE PRODUCING *KLEBSIELLA PNEUMONIAE* BY PHENOTYPIC METHOD

***,[1]Dr. Mohd. Arif, [1]Dr. Dinesh Verma, [2]Dr. A. S. Dalal and [2]Dr. Anamika Vyas**

[1]Department of Microbiology, Government Medical College, Kota, Rajasthan, India 324001
[2]Department of Microbiology, Geetanjali Medical College, Rajasthan, Udaipur, India

ARTICLE INFO

Article History:
Received 20th October, 2016
Received in revised form
09th November, 2016
Accepted 17th December, 2016
Published online 31st January, 2017

Key words:

Carbapenemase,
Klebsiella pneumoniae,
By phenotypic method.

ABSTRACT

Introduction: There are several components of normal microbial flora in human intestine and the second most common aerobic bacterial flora is *Klebsiella species* after *Escherichia* coli. (Podschum *et al*., 1998) *Klebsiella* also accompanies extensive resistance to most of the available antibiotics. Resistance to beta-lactam drugs in gram negative is mainly conferred by beta-lactamase- enzymes that inactivate beta-lactam antibiotics by hydrolysis. (Ghafourian *et al*., 2011) There for this study was designed to determine Carbapenemase production in *Klebsiella pneumonia* from various clinical samples by using phenotypic test (Combined disk test, Modified Hodge test, and (combined disk test +Modified Hodge test both).

Aim and objectives: The present study was undertaken to determine Carbapenemase production in *Klebsiella pneumonia* from various clinical samples by using phenotypic test (Combined disk test, Modified Hodge test, and (combined disk test +Modified Hodge test both).

Materials and Methods: The presnt study was conducted in department of microbiology, Geetanjali medical college Udaipur (raj). Various clinical samples were obtained from the patients who came in various outdoor and indoor department of Geetanjali medical college Udaipur (raj). 100 non duplicates clinical isolates of *Klebsiella pneumonia* were processed for the study. Antibiotic susceptibility testing was performed by Kirby bauer method according to CLSI guidelines and the Imipenem resistant isolates were further tested for carbapenemase production by combined-disk test (CDT) and Modified Hodge test (MHT) (Clinical Laboratory Standards Institute (CLSI), 2014) and (combined disk test +Modified Hodge test both).

Observation and Results: Among 100 *Klebsiella pneumomiae* isolates, 14% resistance for Carbepenems (Imipenem) was observed, and 86% Carbepenems (Imipenem) sensitive. all 14 Imipenem resistant *Klebsiella* pneumoniae isolates, 05 (35.71%) carapenamase positive on CDT; and 04(28.57%) positive by MHT. 03 (21.4)% positive on CDT/MHT whereas 02 (14.28%) were negative on (CDT+MHT) method. Among 14 Imipenem resistant *Klebsiella* pneumonia isolates, 12(85.71%) were carbapenemase producer and 02(14.2%) were non-carbapenemase producer.

Conclusion: To conclude, carbapenemase producing *Klebsiella pneumonia* isolates were relatively high in our institution. Accurate and timely detection of carbapenemase has important implications for efficient infection control and help in reducing the emergence of resistance thus decreases the morbidity and mortality rate.

Citation: Dr. Mohd. Arif, Dr. Dinesh Verma, Dr. A. S. Dalal and Dr. Anamika Vyas, 2017. "Detection of Carbapenemase producing *Klebsiella pneumoniae* by phenotypic method", *International Journal of Current Research*, 9, (01), 45444-45447.

INTRODUCTION

There are several components of normal microbial flora in human intestine and the second most common aerobic bacterial flora is *Klebsiella species* after *Escherichia* coli. (Podschum *et al*., 1998) It is also a leading contributory agent in community acquired and hospital-acquired infections. (Arti Kapil, 9th edition) *Klebsiella* species is in continual association with infections of the urinary and respiratory tracts, as well as soft tissue infections; and can cause fatal septicemia, in neonates. (Henkhoneng Mate and Sulochana Devi, 2014) Along with the persistence cause of wide spectrum infections; *Klebsiella* also accompanies extensive resistance to most of the available antibiotics. Over the time, indiscriminate and irrational use of antibiotics has resulted in development of multi drug resistant strains of the organism. (Agraval *et al*., 2008; Ghafourian *et al*., 2011) Resistance to beta-lactam drugs in gram negative is mainly conferred by beta-lactamases-enzymes that in activate beta-lactam antibiotics by hydrolysis. The most important beta-

****Corresponding author: Dr. Mohd. Arif,***
Department of Microbiology, Government Medical College, Kota, Rajasthan, India 324001

lactamases are extended-spectrum beta-lactamases (ESBLs) and the carbapenemases for example metallo-beta-lactamases (MBLs) (Ghafourian *et al*., 2011). Carbapenemase which have versatile hydrolytic capacities have the ability to hydrolyse penicillins, cephalosporins, monobactams and carbapenems (Favre-Bonte *et al*., 1999). Infections due to *K.pneumoniae* producing the acquired MBLs are on continuous rise. (Paterson *et al*., 2004) There for this study was designed to determine Carbapenemase production in *Klebsiella pneumonia* from various clinical samples by using phenotypic test (Combined disk test, Modified Hodge test, and (combined disk test +Modified Hodge test both).

Aims and Objectives

The present study was undertaken to determin Carbapenamase production in *Klebsiella pneumoniae* from various clinical samples by using phenotypic test (Combined disk test, Modified Hodge test, and (combined disk test +Modified Hodge test both).

MATERIALS AND METHODS

The present study was conducted in department of microbiology, Geetanjali medical college Udaipur (raj).Various clinical samples were obtained from the patients who came in various outdoor and indoor department of Geetanjali medical college Udaipur (raj). 100 non duplicate clinical isolates of *Klebsiella pneumoniae* were processed for the study. Clinical samples mainly included Pus, Sputum, blood, respiratory secretion & Urine samples. All *Klebsiella pneumonia* Isolates were identified by conventional methods. The organism were identified based on Typical Colony morphology, Gram staining, motility and by standard biochemical reactions which include Catalase, Oxidase, Nitrate reduction, Indole, Methyl red, Voges-Proskauer, Citrate, Urease, sugar fermentation test, (Lactose), Aminoacid decarboxylase test (Lysine, Ornithine, Arginine). (BHARTI ARORA, 2012) Antibiotic susceptibility testing was performed by Kirby bauer method according CLSI guidelines and the Imipenem resistant isolates were further tested for carbapenemase production by combined-disc test (CDT) and Modified Hodge test (MHT) and (combined disk test + Modified Hodge test both).

Detection of Carbapenemase production by– CDT

A phenotypic detection method employing combined-disk tests of Imipenem alone and Imipenem-EDTA was evaluated for the detection of carbapenemase production and the differentiation of MBL from KPC enzymes. Escherichia coli ATCC 25922 was cultured overnight and suspended to achieve a 0.5 Mcfarland standard turbidity and was lawn cultured onto a MHA plate using a sterile cotton swab. After drying the disc containing Imipenem (10mcg) and EDTA+Imipenem (10mcg) was placed on plate. The zone diameters of inhibition, EDTA+ Imipenem (10 mcg) 19–21 mm, Imipenem (10mcg) 16–21 mm may indicate carbapenemase production, despite the fact that they are in the old susceptible interpretive categories. For confirmation, perform the MHT. (NOTE: The imipenem disk test performs poorly as a screen for carbapenemases)

Detection of carbapenemase production By– MHT

Escherichia coli ATCC25922 was cultured overnight and suspended to achieve a 0.5 Mcfarland standard turbidity and was lawn cultured onto a MHA plate using a sterile cotton swab. After drying, the disc containing Imipenem (10mcg) was placed at the center of the plate, and an overnight cultured test stain was heavily streaked from the center to the periphery of the plate. The presence of a distorted zone after overnight incubation was interpreted as a positive result. (Clinical Laboratory Standards Institute (CLSI), 2014) MHT positive *Klebsiella pneumomiae* ATCC 1705 and MHT negative *Klebsiella pneumomiae* ATCC 1706 were used for quality control.

RESULTS

The present study was carried out in the department of microbiology, Geetanjali medical college Udaipur (raj). A total of 100 non-repetitive *Klebsiella pneumomiae* isolates obtained from various clinical samples (Pus, Sputum, Blood, Respiratory tract secretions and urine) were included in the study.

Table I. Distribution pattern of *K. pneumoniae* in Clinical samples

Sample	No. of strains isolated	Percentage
Urine	25	25%
Pus	17	17%
ET secretion	18	18%
Sputum	24	24%
Blood	14	14%
Bronchial aspirate	02	2%
Total	100	100%

Table II. Distribution pattern of *K. pneumonia* isolates based on age and gender of the patients

Age (years)	Male	Female	Total (n=100)
< =25	17 (68%)	08 (32%)	25 (25%)
26-35	08 (72.72%)	03 (27.27%)	11(11%)
36-45	14 (87.5%)	02 (12.5%)	16(16%)
46-55	07 (53.84%)	06 (46.15%)	13(13%)
>=56	22 (62.85%)	13(37.14%)	35(35%)
Total	68 (68%)	32 (32%)	100 (100%)

Table III. Distribution pattern of Carbapenems Sensitive and Carbapenems resistant *Klebsiella pneumomiae* isolates in various Clinical samples

Sample	Carbapenems Sensitive	Carbapenem resistant
Urine (n=25)	24 (96%)	01(4%)
Pus (n= 17)	15 (88.23%)	02 (11.76%)
ET secretion(n= 18)	14 (77.77%)	04 (22.22%)
Sputum (n= 24)	22 (91.66%)	02 (8.33%)
Blood (n= 14)	09 (64.28%)	05 (35.71)
Bronchial aspirate (n= 02)	02 (100%)	00
Total (n=100)	86 (86%)	14 (14%)

Table IV. Phenotypic differntiation of clinical isolates *Klebsiella pneumomiae* resistant to carbapenem (Imipenem)

CDT Positive	05
MHT Positive	04
CDT + MHT Positive	03
CDT + MHT Negative	02

Table V. Observation for Carbapenemase producer and non-producers in *Klebsiella pneumomiae* resistant to carbapenem (n=14)

Carbapenemase producer	Carbapenemase Non- producer
12	02

Antibiotic susceptibility testing for a pre-determined panel of antibiotics was performed by Kirby-Bauer disc diffusion method and zone interpretation was done according to CLSI guidelines. Those isolates with reduced susceptibility to carbapenems (Imipenem 16–21 mm or Meropenem) were further screened for the production of carbapenamase by the combined disc method (Maroncle et al., 2002 & Modified Hodge Test (Ørskov *et al*., 1952). In this study, *K. pneumoniae* were predominantly isolated from urine samples (25 %) followed by Sputum samples (24%), ET secretion (18%), pus samples (17%), blood cultures (14%), and Bronchial aspirate (2%) respectively. (Table 1) Among 100 *Klebsiella pneumomiae* isolates; 68 % were obtained from male patients and 32% from females. 62.85% males were in the age group of >=56 years contributed 35% of the total *Klebsiella pneumomiae* isolates. (Table 2) Among 100 *Klebsiella pneumomiae* isolates, 14% resistance for Carbepenem (Imipenem) was observed and 86% Carbepenem (Imipenem) sensitive. (Table 3) All the 14 Imipenem resistant *Klebsiella* pneumonia isolates, were subjected to CDT, 05 (35.71%) of them were MBL producer. On MHT, 04(28.57%) isolates had a clover leaf-like indentation which was considered as MHT positive. 03 (21.4) % of the *Klebsiella* pneumoniae isolates were phenotypically detected as carapenamase producer on both CDT and MHT. No carapenamase activity was detected in 02 (14.28%) of the isolates by either of the methods in combination i.e. (CDT+MHT). (Table 4) The 14 Imipenem resistant *Klebsiella* pneumoniae isolates, 12(85.71%) were carbapenemase producer and 02(14.2%) were non-carbapenemase producer. (Table 5)

DISCUSSION

For multidrug resistant clinical isolates of Enterobacteriaceae, Carbapenems are often considered last resort antibiotics in the treatment of infections. However, during the last decade carbapenem resistance has been increasingly reported among Enterobacteriaceae and is largely attributed to the production of metallo-beta-lactamases (MBLs) and klebsiella pneumonia carbapenemase (KPC). These enzymes efficiently hydrolyse all b-lactams except monobactams. They have shown a worldwide dissemination. KPC and MBL, both carbapenemase have been reported in *Klebsiella pneumonia* from different regions of India. (Leila Azimi *et al*., 2013; Mohammad Shahid *et al*., 2012; Sathya Pandurangan *et al*., 2015; Sanjeev Kumar *et al*.,) In the present study, *K. pneumoniae* were predominantly isolated from urine samples (25 %) followed by Sputum samples (24%), ET secretion (18%), pus samples (17%), blood cultures (14%), and Bronchial aspirate (2%). Similar to our finding; Manikandan *et al*. (2013) also had reported 24% *Klebsiella pneumonia* in sputum. In our study, 25% Klebsiella pneumonia was isolated from urine samples which were also reported by Sarath babu *et al*. (2012). In contrast to our isolation rates, Radhika *et al.* (2014) had reported *K. pneumoniae* 45 % from sputum sample followed by 21% in pus samples, 20% in urine samples, 7% from vaginal and cervical swabs, 4% from bronchial washings, and 3% in blood cultures. In the present study, 68% of culture positive samples were from males and 32% were from female. In studies carried by Sarita Nayak *et al*. (2014) and *Reza Ghotaslou et al.* (2014); both had reported higher culture positive samples in males 68.6% and 59.2% respectively, whereas 31.4% and 40.8% in females. In the current study, 35% subjects were in between the age group of 56-75 years, which is similar to the finding of Gunjal *et al.* (2012) where 40.42% subjects were in >50 year age group. This may be due to the risk of hospital acquired infections due to weaning immunity in old age. In the present study, 14% resistance for Carbepenems (Imipenem) was observed. Similar result was reported by Datta *et al*. (2015) with resistance rate of 12%. In contrast to our finding, much higher rates of carbepenem resistance was reported by Rai, *et al*. (2011) and Kalpana Chauhan, *et al.* with resistance rate of 39% and 29.69, respectively. This may be due to variation in occurance of carbapenemase producers included the study (*Klebsiella pneumonia >E.coli*); partly may be attributed to the study centre and resistance detection method employed. In our study, all the 14 Imipenem resistant *Klebsiella* pneumonia isolates, which were subjected to CDT, 05 (35.71%) of them were MBL producer. On MHT, 04(28.57%) isolates had a clover leaf-like indentation which was considered as MHT positive. 03 (21.4) % of the *Klebsiellae* pneumoniae isolates were phenotypically detected as carapenamase producer on both CDT and MHT. No carapenemase activity was detected in 02 (14.28%) of the isolates by either of the methods in combination i.e. (CDT+MHT). Similarly, Kalpana Chauhan *et al*. had reported, a overall of 106 (29.69%) Imipenem resistance in clinical isolates of *Klebsiella* pneumonia, and were 40 (38.67) % carapenamase positive on CDT; and 34 (32.7%) positive by MHT. 22 (20.75%) positive on CDT/MHT whereas 09(8.49%) were negative on (CDT+MHT) method. Sathya Pandurangan *et al*. (2015) also had reported a total of 43(21.5%) Imipenem resistance, but 11 (25.58) % and 17 (39.53) % were positive on CDT and MHT respectively. On (CDT/MHT) 06 (13.95) % had could be detected as carbapenemase producer. In the present study, of the 14 Imipenem resistant *Klebsiella* pneumonia isolates, 12(85.71%) were carbapenemase producer and 02(14.2%) were non - carbapenemase producer. Kalpana Chauhan *et al*. reported much higher carbapenemase production 97 (91.51%) and 9 (8.4) were non -carbapenemase producer. In phenotypic carbapenemase detection methods- CDT had an advantage over MHT in being less time consuming, technically less demanding, and hence, less cumbersome to perform in routine clinical laboratory such as the case may be for a tertiary care centre like ours. However, supplementing MHT with CDT are rapid and reliable phenotypic tests that can be implemented in routine drug susceptibility testing to detect carbapenemase producing Enterobactericiae. It has an advantage of detecting CRE accurately, timely and cost effectively for better patient outcome, facilitating efficient infection control and reducing the escalation of resistance (Mona fattouh *et al*.).

Conclusion

To conclude, carbapenemase producing *Klebsiella pneumonia* isolates were relatively high in our institution. The significant finding of our study was that 85.71% of *Klebsiella pneumonia* isolates which showed non susceptible zone sizes for carbapenem on disc diffusion test were detected positive (carbapenemase producer) by CDT, MHT and (CDT+MHT). Accurate and timely detection of carbapenemase has important implications for efficient infection control and help in reducing the emergence of resistance thus decreases the morbidity and mortality rate.

REFERENCES

Agraval P, Ghosh AN, Kumar S, Basu B, Kapila K. 2008. Prevelance of extended spectrum of beta lactamase among Escherichia coli and *Klebsiella pneumoniae* in a tertiary

care hospital, *Indian Journal of Pathology and Microbiology,* 51(1):139-42.

Arti Kapil. Ananthanarayan & Paniker's Textbook of Microbiology, *9th edition. Chennai: Universities Press Pvt Ltd.* 2013.

BHARTI ARORA: A text book of practical microbiology,cbs publishers & distributors pvt.ltd. New Delhi; 2012; ISBN: 81239-1405-9, PP43.

Clinical Laboratory Standards Institute (CLSI).Method for Enterobacteriaceae disk diffusion Susceptibility testing of klebsiella pneumoniae ; Approved Guideline eleventh edition CLSI document *M02-A11 (ISBN 1-56238-897-5) Clinical Laboratory Standard Institute, Waync: Pennsylvania19087USA.*

Dr. Ph. Henkhoneng Mate, Dr. Kh. Sulochana Devi, Prevalence of Carbapenem Resistance among Gram-Negative Bacteria in a Tertiary Care Hospital in North-East India Volume 13, Issue 12 Ver. III (Dec. 2014), PP 56-60

Dr. R. Sarathbabu, 2Dr.T.V.Ramani, 3Dr. K.Bhaskara rao, 4 Dr. Supriya Panda, 2012. Antibiotic susceptibility pattern of Klebsiella pneumoniae isolated from sputum, urine and pus samples ISSN : 2278-3008 Volume 1, Issue 2 (May-June), PP 04-09

Favre-Bonte, S., T. R. Licht, C. Forestier, and K. A. Krogfelt. 1999. Klebsiella pneumoniae capsule expression is necessary for colonization of large intestines of streptomycin - treated mice. *Infect. Immun*., 67(11):6152–6156.

Ghafourian S, Sekawi Z, Sadeghifard N, Mohebi R, Neela VK, Maleki A, Hematian A, *et al*. 2011. The Prevalence of ESBLs Producing *Klebsiella pneumoniae* Isolates in Some Major Hospitals, Iran, *The open Microbiology Journal,* 5: 91-95

Kalpana Chauhan, Anita Pandey, Ashish K. Asthana, Molly Madan Evaluation of phenotypic tests for detection of *Klebsiella pneumoniae* carbapenemase and metallo-beta-lactamase in clinical isolates of *Escherichia coli* and *Klebsiella* species IP: 106.215.146.155.

Leila Azimi, Abdolaziz Rastegar Lari, Malihe Talebi, Amirmorteza Ebrahimzadeh Namvar, Somayeh Soleymanzadeh-Moghadam, 2013. Evaluation of Phenotypic Methods for Detection of *Klebsiella Pneumoniae* Carbapenemase-Producing *K. Pneumoniae* in Tehran *J Med Bacteriol., Vol. 2, No. 3, 4, pp. 26-31 jmb.tums.ac.ir*

Manikandan C. and A. Amsath, 2013. Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from urine samples ISSN: 2319-7706 Volume 2 Number 8, pp. 330-337

Maroncle, N., D. Balestrino, C. Rich, and C. Forestier. 2002. Identification of Klebsiella pneumoniae genes involved in intestinal colonization and adhesion using signature-tagged mutagenesis. *Infect. Immun.,* 70(8): 4729–4734.

Mohammad Shahid, Umesh, Farrukh Sobia, Anuradha Singh, Haris M Khan, Abida Malik and Indu Shukla, 2012. Molecular epidemiology of carbapenemresistant Enterobacteriaceae from a North Indian Tertiary Hospital NZ *J Med Lab Science.*

Mona Fattouh, Asmaa Nasr El-din1 and Mohamed A. Omar2 2015. Detection of *Klebsiella pneumoniae* Carbapenemase (KPC) Producing Gram Negative Superbugs: An Emerging Cause of Multidrug-Resistant Infections in General Surgery Department of Sohag University Hospital, Egypt ISSN: 2319-7706 Volume 4 Number 5, pp. 1-15.

Ørskov, I. Nosocomial infections with Klebsiella in lesions of the urinary tract. *Acta Pathol. Microbiol. Scand. Suppl.,* 92:259–271.

Paterson DL, WC KO, Goossens H. 2004. Antibiotic therapy for *Klebsiella pneumoniae* bacteremia. Implication of production of extended spectrum- Lactamases. *J Clin Microbiol.,* 39(5):50

Podschum R, Ullmann U. 1998. Klebsiella spp. As Nosocomial Pathogens: Epidemiology, Taxonomy, Typing methods, and Pathogenicity factors, Clinical Microbiology *Review.* 11(4):589-603

Prasad Gunjal, Shraddha Gunjal. 2012. a cross-sectional study to determine the profile and antibiotic resistance pattern of gram negative bacilli isolated from intensive care unit patients in a tertiary carehospital in ahmednagar, Maharashtra, *IJBAR,* 03(05):282-2012

Priya Datta, Varsha Gupta, Shivani Garg, Jagdish Chander. Phenotypic method for differentiation of carbapenemases in Enterobacteriaceae: Study from north India *IP: 106.215.146.155]*

Radhika B. and I. Jyothipadmaja, 2014. "Sub speciation and antibacterial susceptibility testing of Klebsiella pneumonia" *Pub Health Med Res.,* 2(2):39-4

Rai, S., V Manchanda, NP Singh, IR Kaur, 2011. Zinc-dependent carbapenemases in clinical isolates of family Enterobacteriaceae *Indian Journal of Medical Microbiology,* 29(3): 275-9 52.

Reza Moayednia1, Dariush Shokri2, Sina Mobasherizadeh3, 2014. Frequency assessment of β-lactamase enzymes in Escherichia coli and Klebsiella isolates in patients with urinary tract infection *J Res Med Sci.,* 19:S41-S.

Sanjeev Kumar, Anamika Vyas, S.K. Mehra. Utilization of MacConkey-Meropenem screening Agar for the Detection of Carbapenem Resistanant *Enterobacteriaceae* in a tertiary care hospital, ISSN: 2393 – 9117 47

Sarita Nayak, Suman Singh, Soeb Jankhwala, Riddhi Pradhan. 2014. Prevalence, characterization and clinical significance of *klebsiella pneumoniae* carbapenemase (kpc) producing *klebsiella pneumonia, Int J Med Res Health Sci.,* 3(4):797-803 45.

Sathya Pandurangan, Shamsadh BegumEsak and Anbumani Narayanasamy. 2015. Phenotypic Detection Methods of Carbapenemase Production in Enterobacteriaceae, ISSN: 2319-7706 Volume 4 Number 6, pp. 547-552 44.

****