Int.J.Curr.Microbiol.App.Sci (2018) *7*(1): 594-601

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 01 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.701.072

The Incidence of Carbapenem Resistance in *Klebsiella pneumoniae* Subspecies *pneumoniae* (CRKP) and Antibiotic Susceptibility Profile of *Klebsiella pneumoniae* Subspecies *pneumoniae* strains

Priyanka Singh*

Department of Microbiology, RKDF Medical College and Research Center, Jatkhedi Hosangabad Road, Bhopal-462026, India

**Corresponding author*

Keywords

Klebsiella pneumoniae, CRKP

Article Info

Accepted:
06 December 2017
Available Online:
10 January 2018

A B S T R A C T

Carbapenamases producing *Klebsiella pneumoniae subspp pneumoniae* strains are increasing worldwide and pose a major threat to patient care. Hence, the present study was undertaken to detect the incidence of Carbapenamases producing *Klebsiella pneumoniae subspp pneumoniae* strains isolated from different clinical specimens in the department of Microbiology and Antibiotic Susceptibility profile of *Klebsiella pneumoniae* subspecies *pneumoniae* strains. The study was approved by Institutional Ethical Committee (IEC). The type of study was cross sectional observational study. 300 *Klebsiella pneumoniae subspp pneumoniae* strains were studied. Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method. In our present study, 29.3% strains were CRKP. Out of total 300 *Klebsiella pneumoniae subspp pneumoniae* strains, 83 (27.7%), 90 (30%), 56 (18.7%), 40 (13.3%) and 96 (32%) were positive by Classical Hodge test, Modified Hodge test, MBL confirmatory test, KPC confirmatory test and Carbapenem hydrolysis positive due to MBL and KPC production respectively. In the present study (19/300 i.e.6.3%) *Klebsiella pneumonia* strains were MBL and KPC Co- producers. 13.3% KPC, 18.7% *Klebsiella pneumoniae* strains were MBL producing *Klebsiella pneumoniae* strains were isolated. All 300 *Klebsiella pneumoniae* strains studied were sensitive to colistin (100%), followed by Imipenem (70.6%). All *Klebsiella pneumoniae subspp pneumoniae* strains, isolated in Clinical Microbiology Laboratory should be tested phenotypically for Carbapenamases production.

Introduction

Carbapenamases represent the most versatile family of β- lactamases with broad spectrum of activity unrivaled by other β-lactam-hydrolysing enzymes. They were known as "carbapenamases" as many of them recognize all hydrolysable β- lactams and most are not inhibited by commercially available β-lactamase inhibitors (Walther-Rasmuseen and Hoiby, 2006). Carbapenems are often used as an antibiotic of last resort for treating serious infections caused by multidrug resistant organism. Reduced susceptibility to any carbapenem can be used as screening of carbapenamase. In recent years, carbapenem resistance has emerged in *Klebsiella pneumoniae* isolates due to acquisition of

carbapenamase genes. The production of carbapenamase enzyme has direct carbapenem hydrolyzing activity. Recently Carbapenem resistant pose a real threat to Medical fraternity as the increased frequency with which Enterobacteriaceae cause infection and the increased mortality associated with infections caused by carbapenem resistance.

Metallo betalactamases (MBL) has gained importance in recent years as MBL genes located on integron that resides on mobile genetic elements and can disseminates in hospital Many phenotypic methods have been proposed by CLSI.

Carbapenemases belongs to two major molecular families distinguished by the hydrolytic mechanism at the active sites. Carbapenamases are classified according to their amino acid sequences: Ambler class A (Serine carbapenemases); Class B (Metallo β lactamases); and Class D (OXA carbapenemase).

Classification of carbapenemases

Carbapenemases are divided into two groups according to their active sites:

Serine carbapenemases belonging to the class A Penicillinases and class D Oxacillinases, which contain a serine in the active site and can be inactivated by β-lactamase inhibitors, including clavulanic acid and tazobactam.

Metallo-β-lactamases belonging to the class B carbapenemases, which contain one or more zinc atoms at the active sites, allowing them to hydrolyse the bicyclic β-lactam ring. These enzymes are inhibited by EDTA. (Queenan and Bush K. Carbapenemase, 2007)

Carbapenemases belongs to two major molecular families distinguished by the hydrolytic mechanism at the active sites.

Carbapenamases are classified according to their amino acid sequences

Ambler class A (Serine carbapenemases); Class B (Metallo β lactamases); and Class D (OXA carbapenemase).

Aims and objectives

To study *Klebsiella pneumoniae subspecies pneumoniae* with special reference to carbapenemases production isolated from different clinical specimens in the Department of Microbiology, Jawaharlal Nehru Medical College, Wardha (M.S.).

Materials and Methods

The present study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, Wardha (M.S). The period of study was 2 years from 1.9.2014 to 31.8.2016

IEC clearance

The study was approved by Institutional Ethical Committee. Study was a cross sectional A total number of 300 *Klebsiella pneumoniae subspecies pneumoniae* strains were isolated from different clinical samples, received from the indoor patients departments (IPD) of Acharya Vinoba Bhave Rural Hospital and Jawaharlal Nehru Medical College, Wardha (M.S) which is a tertiary care Hospital in rural setup. In the present study all 300 *Klebsiella pneumoniae subspecies pneumoniae* have been mentioned as *Klebsiella pneumoniae.*

The strains were characterized as *Klebsiella pneumoniae* according to conventional identification tests. (Washington *et al.,* 2006) *Klebsiella pneumoniae* ATCC 700603, and *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were

used as control for all the conventional tests. All antibiotic disks and culture media used in this study were procured from Himedia Laboratories Pvt. Limited, India. Ethylene diamine tetraacetic acid (EDTA) and 3-Aminophenylboronic acid (3-APB), Pheny boronic acid (PBA) were procured from Sigma-Alderich. All E test strips were procured from bioMereux.

All 300 *Klebsiella pneumoniae* strains were screened for Carbapenemase activity by Classical Hodge Test and Modified Hodge Test (MHT). Many phenotypic methods have been proposed by CLSI. The Classical Hodge test (Lee *et al.,* 2003), Modified Hodge Test. (Lee *et al.,* 2001) and Re-Modified Hodge test (Rai *et al.,* 2011) are used for detection and confirmation of Carbapenemase activity. Double disc synergy test and disc potentiation test (Yong *et al.,* 2002) using EDTA are based on this principle. For confirmation many other method used are MBL, E test using Imipenem, Imipenem–EDTA (Walsh *et al.,* 2002) and reduction of MIC of carbapenems in presence of EDTA.

All 300 *Klebsiella pneumoniae* strains were tested for Metallobetalactamases (MBL) production by disc potentiation test and MBL-E test strip (bioMerieux). The MBL-E test is considered as a standard Reference method for MBL detection. (Omair *et al.,* 2012)

Detection of *Klebsiella pneumoniae* carbapenemases (KPC): producing strains was done by combined disc method. Using Imipenem and Phenyl boronic acid in combination the strains indicator used was *Escherichia coli* ATCC 25922.

Classical Hodge test

The indicator organism, *Escherichia coli* ATCC 25922 (turbidity adjusted to 0.5 Mc Farland Standard) was used to inoculate the Mueller Hinton agar plate as lawn culture and the test strain was heavily streaked. Then the plate was allowed to stand for 15 minutes at room temperature and a 10 µg Imipenem disc (HiMedia) was placed at the center. The plate was incubated overnight at 37^0C. The presence of distorted inhibition zone was interpreted as a positive result for Carbapenem hydrolysis screening.

Modified Hodge Test (MHT): (Jarlier *et al.,* 1988)

The broth culture of *Escherichia coli* ATCC 25922 the broth culture of *Escherichia coli* ATCC 25922. Four test strains of *Klebsiella pneumoniae* were streaked, the plate was incubated overnight at 37^0C. The presence of a cloverleaf shape zone of inhibition due to Carbapenemase production by the test strain was considered as positive.

Imipenem- EDTA combined disc test for detection of MBL

In the combined disc test, if the increase in inhibition zone with the Imipenem and EDTA disc was ≥ 7mm than the Imipenem disc alone, it was considered as MBL positive.

MBL E-test

MIC ratio of Imipenem/ Imipenem- EDTA (IP/IPI) of ≥ 8 or deformation of ellipse or phantom zone indicate MBL.

Detection of *Klebsiella pneumoniae* carbapenemases (KPCs): (Tsakris *et al.,* 2011)

KPCs can be mainly detected by combined disk method using Imipenem and Phenyl boronic acid in combination. Lawn culture of test strain (turbidity adjusted to 0.5 Mc Farland) was put on Mueller Hinton (MH) agar and 2 Imipenem (10µg) discs were put

widely apart. To one Imipenem disc Pheny boronic acid (PBA) solution was put, then the MH agar plates were incubated at 37^0C overnight. After incubation, the diameter of growth inhibitory zone was compared between Imipenem disc with Phenyl boronic acid and Imipenem disc alone. The test should be considered positive when growth inhibitory zone around the disc containing Imipenem plus Phenyl boronic acid was ≥ 5mm compared to zone diameter of Imipenem alone.

Detection of metallobetalactamase (MBL) and *Klebsiella pneumoniae* Carbapenemase (KPC): (Fattouh *et al.*, 2015)

In this test, four discs of Imipenem (10µg) alone, Imipenem plus EDTA, Phenyl boronic acid and EDTA in combination were used. The production of both KPC and MBL were considered when the growth inhibitory zone diameter seen around Imipenem disc with both PBA+ EDTA had increased to ≥ 5mm, as compared to the growth inhibitory zone diameter seen around the Imipenem disc alone. When none of the three combined disc tests was positive, the isolate was considered negative for MBL and KPC production.

Results and Discussion

*In MBL and KPC Co-producer strains, four discs IPM/ IPM + EDTA, IPM+ PBA and IPM+EDTA+ PBA were used (Table 1).

Table 1 shows that out of 300 *Klebsiella pneumoniae* strains studied 88 (29.3%) were Imipenem resistant and 212 (70.7%) strains were Imipenem sensitive by Kirby- Bauer disc diffusion method as per CLSI guidelines 2016. All 300 *Klebsiella pneumoniae* strains were screened. for Carbapenem hydrolysis by Classical Hodge Test and Modified Hodge Test. Out of 88 Imipenem resistant strains 78 (88.6 %) were Classical Hodge Test positive and 82 (93.2%) were Modified Hodge Test positive As confirmatory tests for MBL, Disc potentiation test and MBL E-Test were done. Out of 88 Imipenem resistant *Klebsiella pneumoniae* strains 46 (52.3%) were positive by Disc potentiation test for MBL and those 46 were positive by MBL E-Test (Imipenem / Imipenam + EDTA i.e. IP/IPI) the confirmatory test for *Klebsiella pneumoniae* Carbapenemase (KPC) was done by combined disc method using Imipenem / Imipenem plus Phenylboronic acid. Out of 88 Imipenem resistant strains 39 (44.3 %) were positive for KPC by Combined disc methods. Out of 212 Imipenem sensitive strains 5 (5/212 i.e. 2.4%), 7 (3.3%), 10 (4.7%), 1 (0.4%) and 11 (5.2%) strains were positive by Classical Hodge test, Modified Hodge test, MBL confirmatory test (DP test and MBL E- test), KPC confirmatory test and Carbapenem hydrolysis positive due to MBL and KPC production respectively. Hence out of 88 imipenem resistant strains 85 (96.6%) were positive for Carbapenemase production due to MBL and KPC. Out of 212 Imipenem sensitive strains, 11 (5.2%) were positive for Carbapenem hydrolysis due to MBL and KPC production. Out of total 300 *Klebsiella pneumoniae* strains, 83 (27.7%), 90 (30%), 56 (18.7%), 40 (13.3%) and 96 (32%) were positive by Classical Hodge test, Modified Hodge test, MBL confirmatory test, KPC confirmatory test and Carbapenem hydrolysis positive due to MBL and KPC production respectively.

Out of 40 KPC producing strains 20 (50%) strains produced KPC only but 19 (47.5%) strains were MBL and KPC Co- producers and 1 (2.5%) strain

Carbapenemases producing *Klebsiella pneumoniae* strains were detected by Classical Hodge test (CHT) and Modified Hodge test (MHT). The confirmatory tests for MBLs were done by Disc potentiation (DP) test and E test. In 2002, Walsh T R *et al.*, have

reported that for detection of MBL producing strains MBL E test were 100% in agreement with results from PCR and biochemical methods. The confirmatory test for detection of KPC producing strains was done by combined disc method using Imipenem and Imipenem plus Phenyl boronic acid.

The Incidence of Carbapenem Resistant *Klepsiella pneumoniae* (CRKP) strains by various workers. In our present study, 29.3% strains were CRKP. Datta *et al.*, in 2012 reported 43.6% CRKP strains from Delhi. (Datta *et al.*, 2012) As many workers have reported that MBL and KPC genes can be carried by Carbapenem sensitive strains also and if only the Carbapenem resistant strains are studied, the MBL and KPC strains may be missed. Hence in the present study, all 300 *Klebsiella pneumoniae* strains were studied for detection of MBL and KPC. There were 88 (88/300 i.e. 29.3%) Imipenem resistant strains but total Carbapenemase producing (MBL and KPC) strains were 85 (85/88 i.e. 97%). Three (3/88 i.e. 3.4%) were Carbapenemase negative as these three strains were negative by Classical Hodge Test, Modified Hodge Test, and Confirmatory test also. Out of 212 Imipenem sensitive strains, 11 (5.2%) strains were positive for Carbapenemase i.e. 10 MBL and 1 KPC producers were isolated from 212 Imipenem sensitive strains, but no MBL and KPC Co-producer was detected in these 11 strains. In the present study out of 39 KPC producing strains 20 (51.3%) strains produced KPC only and 19 (48.7%) strains were MBL and KPC Co-producers. The MBL and KPC Co-producer strains were detected in the present study by using four discs IPM, IPM plus EDTA, IPM plus Phenyl boronic acid (PBA) and IPM plus EDTA plus Phenyl boronic acid (PBA). As EDTA inhibited MBL, comparing the zone diameter of IPM and IPM plus EDTA plus PBA, the strains producing MBL and KPC both were detected clearly.

In the present study, 18.7% *Klebsiella pneumoniae* strains were MBL producers. Singh *et al.,* from North India have reported as low as 4.3% MBL producing strains. (Singh *et al.*, 2015)

In the present study 13.3% KPC producing *Klebsiella pneumoniae* strains were isolated. Singh *et al.,* in 2015 from North India reported quite high as 41.1% strains and lowest incidence of KPC (1.7%) producing *Klebsiella pneumoniae* strains were reported by (Kumarasamy *et al.*, 2010).

In the present study (19/300 i.e.6.3%) *Klebsiella pneumoniae* strains were MBL and KPC Co- producers. In 2015, Singh *et al.*, reported very high Incidence of 87.5% MBL and KPC producing *Klebsiella pneumoniae* strains.

Figure 1 shows the Antibiotic Suseptibility profile of *Klebsiella pneumoniae* strains. The Highest sensitivity observed was Colistin (100%) followed by Imipenem 70.6%. The *Klebsiella pneumoniae* strains showed lowest sensitivity to Co-Trimoxazole (30.7%) followed by Gentamicin 38.7%. The highest resistant pattern observed was COT, GEN, CIP.

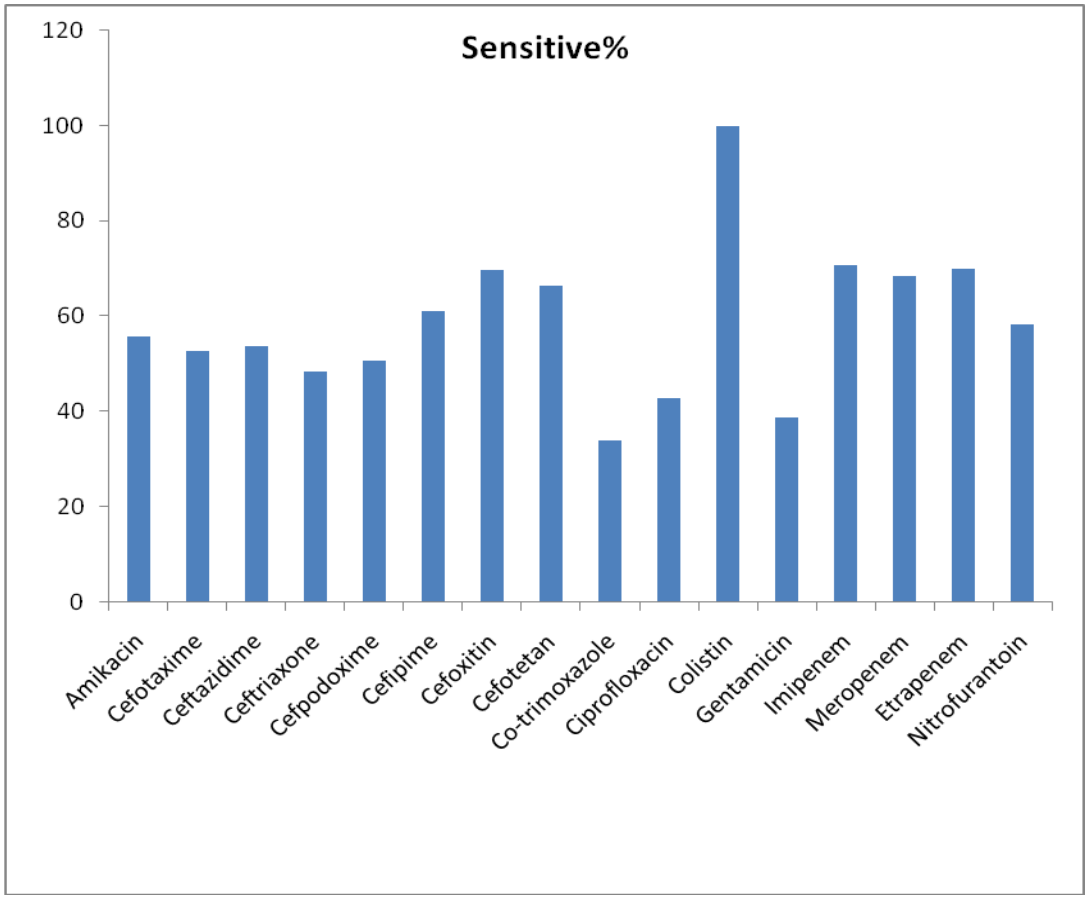
The present study was conducted to detect the incidence of *Klebsiella pneumoniae subspp pneumoniae* strains producing Carbapenemase.

A total number of 300 *Klebsiella pneumoniae* strains isolated from different clinical specimens in the Department of Microbiology, Jawaharlal Nehru Medical College, Wardha (M.S.) were studied. The clinical specimens were received from Indoor Patient Department (IPD) of different clinical specialities of Acharya Vinoba Bhave Rural Hospital and Jawaharlal Nehru Medical College, Wardha (M.S.).

Table.1 Carbapenemase producing *Klebsiella pneumoniae* strains by various methods (n=300)

Klebsiella pneumonia n=300	Screening test for carbapenem hydrolysis		Confirmatory test			Total c carbapenemase producing strains No.
	Classical Hodge test No.	Modified Hodge test No.	MBL		KPC (Combined disc method)	
			DP Test No.	E-Test No.	IPM / IPM + PBA No.	
Imipenem Resistant n= 88	78	82	46	46	39*	85
Imipenem sensitive n= 212	5	7	10	10	1	11
Total n=300	83	90	56	56	40	96

Fig.1 The Antibiotic susceptibility profile of *Klebsiella pneumoniae* strains (n=300)



The rising trend of developing resistance to multiple antibiotics in microorganisms leads to therapeutic failure. Presently antimicrobial resistance is a major threat to patient care and infectious disease control worldwide.

Klebsiella pneumoniae strains can also produce Carbapenemase. Such as Metallo-betalactamases (MBLs) and *Klebsiella pneumoniae* carbapenemases (KPCs) confer resistance to 3rd generation Cephalosporines and Cephamycins etc. which are commonly used for treating patients. Carbapenems are used as a last resort in Carbapenemase producing *Klebsiella pneumoniae* strains and other Enterobacteriaceae and Gram negative bacteria. Carbapenemases such as MBL and KPC producing strains develop resistance to 3rd generation Cephalosporines, Cephamycins and Carbapenems etc. The major problem for Carbapenemase producing strains are, therapeutically available inhibitors are not available and rapid dissemination can occur to other Gram negative bacteria e.g. *Escherichia coli, Pseudomonas aeruginosa*, *Acinetobacter baumanii* etc. in any Health Care set up. Hence, detection of Carbapenemases such as MBL and KPC producing strains are very important for prompt reporting of these strains to clinicians for effective treatment and to take proper infection control measures, to prevent dissemination of these strains in Health care set up.

The present study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, Wardha (M.S.). A total number of 300 *Klebsiella pneumoniae* strains were isolated from different clinical specimens like urine, blood, pus and wound swab etc. and were identified by conventional methods.

The confirmatory phenotypic tests are simple to perform and quite cheap. For detection of Carbapenemase production the E tests are standard reference methods and molecular method like Polymerase chain reaction (PCR) is a gold standard, but both are costly require expertise. Moreover PCR requires tailor made primers and cannot detect the variants.

Hence, to conclude, *Klebsiella pneumoniae* strains which are one of the most common isolates from different clinical specimens must be tested for detection of Carbapenemase by confirmatory phenotypic tests like combined disc methods for KPC and Disc potentiation tests for MBL by Clinical Microbiology Laboratory to prevent the delay in detection of these Carbapenemase producing strains to get a good therapeutic outcome for the patients and to prevent the spread of these β-lactamases producing strains in the Health care set up by taking proper Infection control measures.

References

Datta P, Gupta V, Shivani G. Phenotypic method for differentiation of carbapenemases in Enterobacteriaceae. Study from North India. Indian J Pathol Microbiol. 2012; 55(3): 357-360.

Fattouh M, Nasr El- din A, Omar² Mohamed A. Detection of Klebsiella pneumonia Carbapenemase (KPC) producing Gram Negative Superbugs: An Emerging cause of Multidrug-resistant Infection in General surgery Department of Sohag University Hospital, Egypt. Int. J. Curr. Microbiol. App. Sci. 2015; 4(5): 1-15.

Jarlier V *et al.*, extended spectrum beta-lactamase conferring resistance to newer β-lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns. Rev Infect Dis. 1988; 10: 867-878.

Kumarasamy K K, Toleman M A, Walsh T R, Bagaria J, Butt F, Balakrishnan R *et al.*, Emergence of a new antibiotics resistance mechanism in India, Pakistan

and the U.K. A molecular, biological and epidemiological study. Lancet mInfect Dis. 2010; 10: 597-602.

Lee K, Chong Y, Shin H B, Shin Y A, Kim D, Yong, Yum J H. Modified Hodge and EDTA disc synergy test to screen metallo beta-lactamases producing strains of Pseudomonas spp. and Acinetobacter spp. Clin Microbiol Infect. 2001; 7: 88-91.

Lee K, Lim Y S, Yong D *et al.,* Evalution of Hodge test and Imipenem–EDTA double disc synergy test for differentiation of metallo beta-lactamases producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol. 2003; 41: 4623-4629.

Omair M, Usman J, Kaleem F *et al.,* Evalution of Combined disc methods for detection of metallo- betalactamase producing Gram negative bacilli. Malaysian Jr. of Microbiol. 2012: 8 (1): 21-25.

Queenan A M, Bush K. Carbapenemase: the versatile β-lactamases. Clin Microbiol Rev. 2007; 20: 440-458.

Rai S, Manchanda V, Sigh N P *et al.,* Zinc dependent carbapenemases in clinical isolates of family Enterobacteriaceae. Indian J. of Medical Microbiol. 2011; 29(3): 275-279.

Singh M, Kakati B, Agrarwal R K, Aarti Kotwal. Detection of Klebsiella pneumoniae Carbapenemases (KPC) among ESBL/ MBL producing Clinical Isolates of Klebsiella pneumonia. International Journal of Current Microbiology and applied Sciences 2015; 4(4): 726-731.

Tsakris A, Digalaki K T, Poulou A *et al.,* Comparative evaluation of combined disc tests using different boronic acid compounds for detection of Klebsiella pneumoniae carbapenemase- producing Enterobacteriaceae clinical isolates. J Clin Microbiol. 2011; 49(8): 2804-2809.

Walsh T R, Bolmstrom A, Qwarnstrom A *et al.,* Evolution of new E test for detecting metallo betalactamases in routine clinical testing. J Clin Microbiol. 2002; 40: 2755-2759.

Walther-Rasmuseen J, Hoiby N. OXA- type Carbapenemases. Journal Antimicrob Chemothers. 2006; 57: 373-378.

Washington C W Jr, Stephen DA, Williams M J, Elmer W K, Gary W P, Paul C S, Gail L W. Enterobacteriaceae.in chapter 6. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed, Lippincott Williams & Wilkins, Philadelphia, USA, 2006; pg.211-302.

Yong D, Lee K, Yum J H, Shin H B, Rossolini G M, Chong Y. Imipenem–EDTA Disc method for differentiation of metallo betalactamases producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol. 2002; 40: 3798-3801.

How to cite this article:

Priyanka Singh. 2018. The Incidence of Carbapenem Resistance in *Klebsiella pneumoniae* Subspecies *pneumoniae* (CRKP) and Antibiotic Susceptibility Profile of *Klebsiella pneumoniae* Subspecies *pneumoniae* strains. *Int.J.Curr.Microbiol.App.Sci.* 7(01): 594-601.
doi: https://doi.org/10.20546/ijcmas.2018.701.072