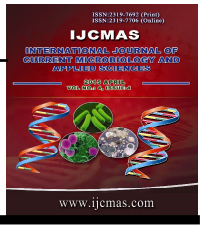
Int.J.Curr.Microbiol.App.Sci (2015) 4(4): 726-731

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 4 (2015) pp. 726-731**
http://www.ijcmas.com



Original Research Article

Detection of *Klebsiella pneumoniae* carbapenemases (KPCs) among ESBL / MBL producing clinical isolates of *Klebsiella pneumoniae*

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Keywords

Klebsiella pneumonia, extended-spectrum - lactamases (ESBLs), MBL producing clinical isolates

A B S T R A C T

Klebsiella pneumoniae is an established pathogen responsible for hospital acquired infections. The spread of *Klebsiella pneumoniae* harbouring extended-spectrum - lactamases (ESBLs) has become worldwide concern, leaving the carbapenems as the only therapeutic option. However, carbapenem-resistant *Klebsiella pneumoniae*, has emerged as an important challenge in health-care settings. The aim of our study was to detect the prevalence of *Klebsiella pneumoniae* carbapenemase (KPC) and to study their Antibiotic sensitivity pattern in this subhimalayan region by using phenotypic methods. A total of 184 *Klebsiella pneumoniae* isolates were obtained from various clinical samples of which 65 (35.32%) were detected ESBL by Double disc synergy test and 8 (4.34%) MBL by Combined Disc Test. Out of these 73 ESBL and MBL producing isolates 30 (41.1%) were found to produce KPC by boronic acid test. Antibiotic susceptibility pattern of the KPC producing isolates were found to be more resistant than the non KPC producing isolates. However, both were found to be highest susceptible to Polymyxin B followed by tigecycline. This simple test with boronic acid if included by clinical microbiology laboratories into their daily routine can detect KPC producers which can be crucial to limit their spread and ensuring an optimal clinical outcome.

Introduction

Klebsiella pneumoniae is one of the most important gram negative bacterial pathogen which has caused worldwide concern because of its association with life-threatening nosocomial infections and its multidrug resistant (MDR) property. Owing to its ability to produce extended –spectrum β-lactamases (ESBL), carbapenems have become the preferred antimicrobial for treating such conditions which in turn has resulted in emergence of carbapenem resistant strains (Falagas ME et al, 2007).

Resistance to carbapenems is mostly due to production of enzymes - Carbapenemases, which are divided into Ambler Classes A, B and D. Class A (serine carbapenemase) enzymes include enzymes such as KPC, IMI, SME, etc and are commonly present in members of Enterobacteriaceae. KPCs are

usually found in *Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli, Citrobacter* species and *Enterobacter* species (Datta P et al, 2012).

The main challenge in identification of isolates harbouring KPCs in clinical laboratories is that the presence of a KPC does not always result in high level resistance to carbapenems, but may cause MIC elevations that remain within the susceptible or intermediate range. These increased MICs if goes unnoticed can result in therapeutic failure. So it is of utmost importance to detect KPC especially in those organisms which are already resistant to commonly used antibiotics. Though gold standard for confirmation of KPC is PCR of bla kpc gene, it is time consuming and requires isolates to be sent to reference laboratories, phenotypic methods can be employed to detect the presence of KPC in clinical microbiological laboratories (Hirsch E.B et al, 2010).

The prevalence of KPC production among *Klebsiella pneumoniae* harbouring ESBL and MBL would have important implications in selection of adequate initial empiric therapy for these conditions and to preserve the activity of available antibiotics. Hence this study was carried out with an aim to identify KPC phenotypically among the ESBL and MBL producing *Klebsiella pneumoniae* isolates and to determine their antibiotic susceptibility pattern.

Material and Methods

The study was a prospective one carried out in the Department of Microbiology, Himalayan Institute of Medical Sciences, Dehradun in North India. All *Klebsiella pneumoniae* isolates obtained from various from clinical samples like urine, pus, wound swabs, body fluids, sputum, throat swab and endotracheal secretions over a period of one year were included in the study. The bacterial isolates were identified according to standard microbiological procedure (Forbes BA et al, 2007). Antimicrobial susceptibilities of all the isolates were determined by disc diffusion method as per CLSI guidelines on Muller Hinton agar plate (Miles RS et al, 2008).

Production of ESBL among the isolates were detected by double disc synergy test (Clinical and Laboratory Standards Institute, 2007) and MBL detection was done by combined disc test (Clinical and Laboratory Standards Institute, 2007).The detection of KPC production was performed by phenyl boronic acid among the ESBL and/or MBL producers (Doi Y et al, 2008)

Phenotypic detection of KPC

The stock solution was prepared by dissolving phenylboronic acid in dimethyl sulfoxide at a concentration of 20 mg/ml. From this solution, 20µl (containing 400µg of boronic acid) was dispensed onto commercially available antibiotic disk. The disk was then dried and used within 60 mins. The tests were performed by inoculating Muller-Hinton agar by the standard diffusion methods (Doi Y et al, 2008). Disks containing meropenem and cefepime were placed with and without boronic acid onto the agar. The agar plates were then incubated at 37^{o} C overnight.The diameter of the growth-inhibitory zone around the corresponding beta lactam disk with boronic acid was compared with that around the corresponding beta lactam disk without boronic acid. The test were considered positive for the production of KPC enzyme when the diameter of the growth – inhibitory zone around a beta lactam disk with boronic acid was ≥5mm larger than that around a disk containing the

beta lactam substrate alone (Doi Y et al, 2008).

Result and Discussion

A total of 184 *Klebsiella pneumoniae* isolates were obtained from various clinical samples of which 65 (35.32%) were ESBL and 8 (4.34%) MBL producers. Out of the 65 isolates of ESBL producing *K. pneumoniae*, maximum number were recovered from urine (41.5%), followed by pus, ET Secretion, sputum (20.0%, 13.8%, 9.2% respectively) and others (central line tip, ICD drain, Throat swab, TT secretion) 1.5% each. The maximum numbers of MBL producing isolates were recovered from ET secretion (62.5%). Out of these 73 ESBL and MBL producing isolates 30 (41.1%) were found to produce KPC by boronic acid test. (Table 1)

The antimicrobial susceptibility pattern of the isolates revealed that the KPC producing isolates were more resistant than the non KPC producing isolates. Maximum sensitivity of the KPC producing isolates were exhibited to Polymyxin B (93.3%) followed by Tigecycline (86.7%) (Table 02)**.**

The emergence and rapid dissemination of KPC producing *K.pneumoniae* has now become a global health threat. The genes coding for KPC enzymes are located on the plasmids which can transmit the resistance between the organisms of the same and different species & also contribute to the expression and dissemination of the β-lactam resistant trait (Kumarasamy K K et al, 2010). Due to the fact that these enzymes confer various levels of resistance to all β-lactams, including carbapenems and the limitation in the laboratory detection methods, the delay in the microbiological laboratory confirmatory results often occur (Arnold RS et al, 2011). Therefore, infection by KPC may account for an increase in the rate of morbidity and mortality as compared to infection by carbapenem susceptible Enterobacteriaceae (Centers for Disease Control and Prevention (CDC), 2009).

In our study, out of 184 *K. pneumoniae*, 35.32% isolates were found to be ESBL producers by Double disc synergy test and 4.34% were MBL producer by Combined Disc Test (CDT). In a study conducted in a Tertiary care hospital by Hosoglu et al also reported 30.18% of ESBL producing *K. pneumoniae* among clinical isolates (Hos¸o˘glu S et al, 2007). Similar rates were also reported from Chennai in 2005 where 23.6% of ESBL producing *K. pneumoniae* were isolated from clinical isolates (Ananthan S et al, 2005). In another study done in north India, reported 5.75% of MBL production in their health care set up (Datta P et al, 2012).

This variation in the rates of drug resistance may be due to various factors like existing antibiotic policy and frequent use of higher generation beta lactams in critically ill patients in different hospitals in different regions.

In this study, out of the total ESBL producing *Klebsiella* isolates, maximum were isolated from urine (41.5%), followed by pus (20%) and ET secretion (13.8%). The reason for this outcome might be due to the large number of urine samples in the lab during the study period and most of the cases were from IPD and were catheterized. Similarly, in a study done in Saudi Arabia, 57.5% of the ESBL producing *Klebsiella pneumoniae* were isolated from urine samples and 11.32% were isolated from pus samples (Kader A A et al, 2005).

The maximum MBL producing isolates were recovered from ET secretion (62.5%)

in our study which may be due to the fact that the patients with ET tube were from ICU and ICUs are the epicenter for spawning multidrug resistance within hospitals.

Out of the 73 ESBL and MBL producing isolates 30 (41.1%) were found to produce KPC by boronic acid test in our study. A study from Bangladesh revealed that 4.79% of *K. pneumoniae* were KPC positive whereas only 1.70% KPC positive *K. pneumoniae* isolates were identified in one Indian study (Kumarasamy, K. K., et al. 2010).

High prevalence of KPC producing isolates in our study might be due the fact that we have performed boronic acid test only in ESBL and MBL producing isolates. Tsakris et al have also reported that 48 of the 57 KPC bearing isolates were also ESBL producers (Tsakaris A et al, 2009).

In our study, all of the KPC producing isolates were found resistant to amoxycillin, cefadroxil, ceftriaxone, ceftazidime. Besides these, higher resistance was also found to cotrimoxazole (96.7%), amoxy-clav (93.3%) & ticarcillin–clavulanate (90%). Twenty eight (93.3%) of the KPC producing *K. pneumoniae* were found sensitive to polymyxin B, followed by tigecycline (86.7%). Similarly, in a study done in Bangladesh by Hayder et al, 100% resistance was reported to cefepime, Ceftriaxone, amoxicillin, ciprofloxacin, ampicillin, chloramphenicol and PIT. Whereas, higher resistance was also noted to gentamicin (93.5%), amikacin (96.8%), cotimoxazole (93.5%) in the same study. However, they found that all KPC (100%) were sensitive against polymyxin B (Hayder N et al, 2012). Out of the total Non- KPC producing isolates among ESBL and MBL producers, none of them were found sensitive to amoxycillin, cefadroxil, ceftriaxone, ceftazidime whereas, 97.7% were found to be sensitive to polymyxin B, followed by imipenem (95.3%). The test of significance for the effect of Imipenem on the KPC producers and Non KPC producers showed the P value of 0.002, which was significant. Overall, KPC non-producing bacteria showed higher sensitivity rates to all groups of antibiotics as compared to KPC producers.

Screening with boronic acid disk may detect KPC producers among *K. pneumoniae* isolates that exhibit reduced susceptibility to either carbapenems or expanded spectrum cephalosporins which may otherwise go unnoticed during routine susceptibility testing. This simple test if included by clinical microbiology laboratories into their daily routine can detect KPC producers which can be crucial to limit their spread and ensuring an optimal clinical outcome.

Table.1 KPC production among ESBL and MBL producing Isolates by Phenyl boronic acid test (PBA) (n=73)

Isolate	Total Isolates	KPC producer	NonKPC producers	P value
ESBL	65	23 (35.4%)	42 (64.6%)	
MBL	8	7(87.5%)	1 (12.5%)	
Total	73	30 (41.1%)	43(58.9%)	P=0.004

Table no.1 shows that 35.4% of the ESBL producers were found to produce KPC enzyme, whereas, 87.5% of MBL were found to produce KPC enzyme. This proportion was statistically significant at 5% level of significance

Table.2 Comparison of antimicrobial susceptibility pattern of KPC producing and non KPC producing isolates

Antibiotics	**KPC producing isolates(S%)**	**Non KPC producing isolates (S%)**	**P- value**
Gentamicin (10 µg)	10 (33.3%)	19 (44.2%)	0.4667
Amikacin (30 µg)	18 (60.0%)	31 (72.1%)	0.3183
Tobramycin (10 µg)	14 (46.7%)	29 (67.4%)	0.0938
Netilimicin (30 µg)	19 (63.3%)	32 (74.4%)	0.437
Amoxycillin (25 µg)	0 (0.0%)	0 (0.0%)	1.0000
Cefadroxil (30 µg)	0 (0.0%)	0 (0.0%)	1.0000
Ceftriaxone (30 µg)	0 (0.0%)	0 (0.0%)	1.0000
Ceftazidime (30 µg)	0 (0.0%)	0 (0.0%)	1.0000
Cefepime (30 µg)	6 (20.0%)	13 (30.2%)	0.4200
Amoxy-clav (30 µg)	2 (6.7%)	4 (9.3%)	1.0000
Tetracycline (30 µg)	10 (33.3%)	13 (30.2%)	0.8027
Cotrimoxazole (25 µg)	1 (3.3%)	2 (4.7%)	1.0000
Ciprofloxacin (5 µg)	7 (23.3%)	16 (37.2%)	0.3060
Ofloxacin (5 µg)	9 (30.0%)	21 (48.8%)	0.1477
Levofloxacin (5 µg)	9 (30.0%)	21 (48.8%)	0.1477
Piperacillin-Tazobactum (100/10 µg)	19 (63.3%)	36 (83.7%)	0.0575
Ticarcillin-clavulanate (75/10 µg)	3 (10.0%)	2 (4.7%)	0.6425
CFS (50/50 µg)	20 (66.7%)	36 (83.7%)	0.1014
Chloramphenicol (30 µg)	16 (53.3%)	32 (74.4%)	0.08
Imipenem (10 µg)	20 (66.6%)	41 (95.3%)	0.002
Tigecycline (15 µg)	26 (86.7%)	28 (65.1%)	0.0572
Ceftazidime-clavulanic acid (30/10 µg)	22 (73.3%)	37 (86.0%)	0.2302
Polymyxin B	28 (93.3%)	42 (97.7%)	0.5644

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