

Original Research Article

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Detection of Metallo-Beta-Lactamase Production among *Klebsiella pneumoniae* Isolates by Phenotypic Detection Methods

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ABSTRACT

Resistance to carbapenems is an emerging problem among *Klebsiella pneumoniae* isolates. A simple and effective phenotypic detection method for carbapenemase production is required in health care settings. A total of 100 clinical isolates of *Klebsiella pneumoniae* from various samples were screened for imipenem resistance. The imipenem resistant strains were further tested for metallo-beta-lactamase (MBL) production by imipenem combined disc test and ceftazidime combined disc test. Among the 100 isolates, 62 isolates were imipenem resistant and of these 45 were MBL producers. Ceftazidime combined disc method alone could detect 24 (53.3%), imipenem combined disc method alone could detect 12 (26.6%) and nine (20%) of the isolates were positive by both these methods. The present study highlights the burden of MBL producing *Klebsiella pneumoniae* in a North Indian tertiary care hospital. The study findings suggest that using ceftazidime combined disc test alone had a better yield of MBL when compared to imipenem combined disc test alone. However, combination of both the methods resulted in greater detection of MBL production.

Keywords

Metallo-beta-lactamases, *Klebsiella pneumoniae*, Ceftazidime combined disc test, Imipenem combined disc test

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Introduction

Klebsiella pneumoniae is a highly virulent organism, known to spread multidrug resistance in hospital setups. (Won SY *et al.*, 2011) *Klebsiella pneumoniae* is responsible for hospital acquired urinary tract infections, bacteremia, pneumonia and intra-abdominal infections. (Lim SM *et al.*, 2005) Multidrug resistant *Klebsiella* spp. is one of the major reasons for mortality and morbidity among hospitalized patients.

The increased use of carbapenems leads to emergence of the resistant strains and their

outbreak in hospital setting. (Khajuria A *et al.*, 2014) Resistance to carbapenems which are one of the last line in the management of infection is now emerging at an alarming rate. Acquired metallo-beta-lactamase is one of the worrisome resistance mechanisms owing to their capacity to hydrolyze the beta lactam class of antimicrobial agents with the exception of aztreonam. Since their genes are carried on a highly mobile element, it thus allows for their easy dissemination. (Walsh TR *et al.*, 2005)

Resistance to carbapenems is an emerging problem among *Klebsiella pneumoniae*

isolates. Phenotypic detection methods for carbapenemase detection are the need of the hour. Hence, the present study was conducted to explore the various phenotypic methods for the detection of metallo-beta-lactamase production among clinical isolates of *Klebsiella pneumoniae*.

Materials and Methods

This cross sectional study was conducted in Department of Microbiology, Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India. A total of 100 clinical isolates of *Klebsiella pneumoniae* from various clinical samples (sputum, pus, blood, urine and high vaginal swabs) were included in the study. The antibiotic susceptibility testing was done on the isolates as per CLSI guidelines. (CLSI 2014)

Combined disc methods

Isolates showing reduced susceptibility to imipenem were further processed for MBL production by zone enhancement with EDTA impregnated imipenem and ceftazidime discs. Test organisms were impregnated on MHA plate. Two 10µg imipenem discs and two 30µg ceftazidime discs were placed on the inoculated plate and to one imipenem and ceftazidime disc 750µg of EDTA was added. The plates were incubated at 35°C for 16-18 hours. Zone size enhancement of ≥ 7 mm for ceftazidime EDTA disc compared to ceftazidime alone and a zone enhancement of ≥ 7 mm for imipenem EDTA disc compared to imipenem disc alone was taken as a positive criterion for MBL production (Yong *et al.*, 2002; Galani *et al.*, 2008)

Statistical analysis

The data was collected and analysed by using Chi-square test. A 'p' value of <0.05 was considered as statistically significant and p

value of < 0.001 was considered as highly significant.

Results and Discussion

The study involved a total of 100 consecutive, clinical isolates of *Klebsiella pneumoniae* from various clinical samples for a period of one year. Infection were detected in blood(41%), followed by pus(27%), urine(26%), sputum (4%) and high vaginal swabs (2%). A total of 80 (80%) isolates were from inpatients and 20 (20%) were from outpatients. Table 1 shows the sample wise distribution of *K. pneumoniae* among indoor and outdoor patients.

Susceptibility pattern of the isolates

The susceptibility pattern of isolates among metallo-beta-lactamase producers and non-metallo-beta-lactamase producers is mentioned in Table 2. Among the *Klebsiella pneumoniae* isolates highest resistance was for cephalosporins. Second generation cephalosporins, cefoxitin and cefuroxime showing 93% resistance, third generation cephalosporins that is ceftazidime 94%, cefotaxime 93% and ceftriaxone 83% and fourth generation cephalosporin cefepime showing 95% resistance, respectively.

Among the carbapenems least resistance was for ertapenem 56%, followed by imipenem 62% and meropenem 70% (Table 2). Resistance to imipenem and meropenem was more among MBL producers than non MBL producers and was found to be statistically highly significant. (p value <0.001). Reduced susceptibility to imipenem was shown by 62 isolates and these were further processed for metallo-beta-lactamase production.

Metallo-beta-lactamase detection was done by combined disc method using imipenem and ceftazidime disc.

Table.1 Distribution of *Klebsiella pneumoniae* isolates among indoor and outdoor patients

Type of sample	k. pneumoniae strains isolated in indoor patients n=80		k. pneumoniae strains isolated in outdoor patients n=20		Total	
	No.	%	No.	%	No.	%
Blood	40	50	1	5	41	41
Pus	25	31.2	2	10	27	27
Urine	10	12.5	16	80	26	26
Sputum	3	3.75	1	5	4	4
HVS*	2	2.5	0	0	2	2
TOTAL	80	100	20	100	100	100

*HVS- high vaginal swabs

Table.2 Comparison of antimicrobial resistance pattern of MBL and non MBL producing *K. pneumoniae* strains to various antibiotics

Antimicrobial drugs	Resistant isolates		MBL producer N=45		MBL non producer N=55		P value
	No.	%	No.	%	No.	%	
β-lactams							
Ampicillin	87	87	38	84.4	49	89	0.6976
ceftazidime	94	94	43	95.5	51	92.7	0.8656
cefepime	95	95	42	93.3	53	96.3	0.8176
ceftriaxone	83	83	41	91.1	42	76.3	0.0919
cefoxitin	93	93	44	97.7	49	89	0.1936
cefuroxime	93	93	43	95.5	50	90.9	0.6086
cefotaxime	93	93	43	95.5	50	90.9	0.6086
aztreonam	82	82	36	80	46	83.6	0.8342
imipenem	62	62	45	100	17	30.9	0.0001
meropenem	70	70	44	97.7	26	47.2	0.0001
ertapenem	56	56	30	66.6	26	47.2	0.0816
Amoxicillin-clavulanic acid	84	84	36	80	48	87.2	0.4760
Ampicillin sulbactam	88	88	42	93.3	46	83.6	0.2399
Ticarcillin clavulanic acid	90	90	40	88.8	50	90.9	0.7376
Piperacillin tazobactam	77	77	39	86.6	38	69	0.0659
Fluoroquinolones							
ciprofloxacin	83	83	38	84.4	45	81.8	0.9360
levofloxacin	84	84	39	86.6	45	81.8	0.7011
Aminoglycosides							
Amikacin	75	75	35	77.7	40	72.7	0.7277
gentamicin	83	83	41	91.1	42	76.3	0.0919
Others							
Trimethoprim sulfamethoxazole	81	81	40	88.8	41	74.5	0.1181

Table.3 Evaluation of combined disc method using imipenem and ceftazidime disc for detection of MBL production

Method	Number	percentage
MBL producer by CDT using imipenem disc alone	12	26.6
MBL producer by CDT using ceftazidime disc alone	24	53.3
MBL producer by CDT using both imipenem and ceftazidime disc	9	20
Total MBL producer	45	100

Table 3 compares the combined disc method using imipenem and ceftazidime disc test. Ceftazidime combined disc method alone could detect 24(53.3%), imipenem combined disc method alone could detect 12 (26.6%) and nine (20%) of the isolates were positive by both these methods.

The present study found highest resistance was for cephalosporins and least resistance for carbapenems among clinical isolates of *Klebsiella pneumoniae*. The study also highlights that ceftazidime combined disc test was a better method for metallo-beta-lactamase detection compared to imipenem combined disc test.

Rampant use of antimicrobial agents often leads to emergence of multidrug resistant *Klebsiella pneumoniae* isolates. Emergence of these multidrug resistant pathogens leads to an increasing need for newer antimicrobial discoveries. With only a few drugs left for treatment of the isolates we will reach a dead end soon (George PC *et al.*, 2008). Infections caused by these resistant strains leads to prolonged hospital stay and increased health care cost for patients. (Nordmann P *et al.*, 2009).

In the current study resistance to cephalosporins like cefoxitin, cefuroxime, cefotaxime, ceftriaxone, ceftazidime and cefepime was 93%, 93%, 93%, 83%, 94% and 95%. The resistance pattern is lower in study done by Kumar where he have observed 47.7% resistance to cefepime, 76.4%

resistance to cefuroxime and 52.8% resistance to cefotaxime (Kumar, 2013). Mehrgan *et al.*, had shown 84.7% resistance to ceftazidime and 86.6% resistance to cefotaxime which are comparable to present study. (Mehrgan *et al.*, 2010) Resistance to amikacin and gentamicin was 75% and 83% respectively in the present study. Comparable results were seen in a study by Mehrgan *et al.*, who showed 72.8% and 78.2% respectively (Mehrgan *et al.*, 2010). The difference in antimicrobial susceptibility could be due to varied geographical location and the commonly used antimicrobials in the setup.

The rate of MBL production ranges from 9.5-100%. In the present study MBL production was 45%. Studies done in North India showed a rate of 9.5% and that in Central India showed a rate of 100%. (Seema K *et al.*, 2011; Bora A *et al.*, 2014; Khajuria A *et al.*, 2014). This variation could be due to use of difference in the use of carbapenems in the setup.

Resistance to MBL producers were more compared to non MBL producers for antibiotics ceftriaxone (91.1%, 76.3%), cefoxitin (97.7%, 89%), imipenem (100%, 30.9%), meropenem (97.7%, 47.2%), piperacillin-tazobactam (86.6%, 69%), gentamicin (91.1%, 76.3%) and cotrimoxazole (88.8%, 74.5%). Resistance to imipenem and meropenem was more among MBL producers than non MBL producers and was found to be statistically highly significant. (p value <0.001)

Combined disc method using ceftazidime combined disc alone could detect 24(53.3%) compared to 12(26.6%) (46.7%) by imipenem combined disc test alone in the current study. Nine isolates were MBL producers by both the methods. Pandya *et al.*, in their study found imipenem combined disc test could detect 96.3% compared to ceftazidime combined disc test (85.19%). (Pandya NP *et al.*, 2011) Though our study shows ceftazidime combined disc method a better method, more studies are required to rationalize this finding.

The present study highlights the burden of MBL producing *Klebsiella pneumoniae* in a North Indian tertiary care hospital. Phenotypic detection methods are required in health care centers as genotyping would not be feasible in most healthcare setting. We believe that combination of both the methods resulted in greater detection of MBL production. A simple and inexpensive phenotypic method would help in rapid identification of MBL producer. Lastly, a combined effort of clinicians and microbiologist with a good antibiotic stewardship in hospitals will help in preventing the spread of these resistant bug.

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