LIST OF PUBLICATIONS

1. Experience with a commercial assay for detecting different carbapenemases in Enterobacteriaceae – International Journal of Current Microbiology and Applied Sciences 2015; 4 (3): 150-156
2. Comparative evaluation of rapid colorimetric tests for detecting carbapenemase enzymes in Gram-negative bacilli –Indian Journal of Microbiology Research 2017; 4(3): 263-266.
3. Universal presence of blaNDM-1 in carbapenem-resistant Gram-negative bacilli in an Indian hospital in 2015 –Journal of Clinical and Diagnostic Research 2017; 11(9): 1-3.

LIST OF PRESENTATIONS

1. Experience with a commercial assay for detecting different carbapenemases in Enterobacteriaceae – National Symposium on Hospital Acquired infections and Antimicrobial Resistance at Aligarh Muslim University in December 2014.
2. Evaluation of commercial assay for rapid detection of carbapenemases in Enterobacteriaceae and *P. aeruginosa.* - National CME cum MICROCON North-West Chapter at Sri Guru Ram Das Institute of Medical Sciences, Amritsar in April 2016
3. Characterization of carbapenem resistance by Phenotypic and Genotypic methods at a Tertiary care centre in Uttarakhand. – UP-UK MICROCON at Himalayan Institute of Medical Sciences, Jolly Grant in February 2017.

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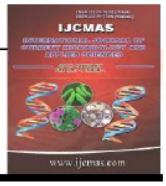

Original Research Article

Experience with a Commercial Assay for Detecting Different Carbapenemases in Enterobacteriaceae

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K e y w o r d s

Carbapenem resistance, Enterobacteriaceae, North India,

Rosco KPC+MBL kit, Class A and Class B carbapenemases

A B S T R A C T

Metallo- β - lactamases and serine carbapenemases in Enterobacteriaceae are rapidly threatening the utility of carbapenems. This has led to the need for specific diagnostic tests to detect these enzymes with high sensitivity and to discriminate between them. Reliable detection of carbapenemases is necessary to implement contact precautions and for outbreak detection. Our aim was to detect Class A Klebsiella pneumoniae carbapenemase (KPC) and similar serine carbapenemases, Class B metallo-beta-lactamases (MBL), and AmpC-type beta-lactamases in carbapenem-resistant Enterobacteriaceae isolates in our teaching hospital, using the proprietary KPC + MBL Detection Kit from Rosco Diagnostica, Denmark. Fifty meropenem-resistant strains isolated at Himalayan Institute of Medical Sciences, Dehradun between November 2013 and June 2014, were analysed with the Rosco KPC + MBL Kit comprising of four disks: meropenem 10 µg (to confirm resistance), meropenem + phenylboronic acid (inhibitor of KPC-type carbapenemases and AmpC-type beta-lactamases), meropenem + dipicolinic acid (inhibitor of MBLs), and meropenem + cloxacillin (inhibitor of AmpC-type beta-lactamases). The dominant species were *Klebsiella pneumoniae* with 22 (44%), *Escherichia coli* with 10 (20%) and *Enterobacter cloacae* with seven (14%) isolates. The remaining isolates included *Enterobacter aerogenes, Citrobacter freundii*, *Citrobacter koseri*, *Serratia marcescens, Proteus vulgaris and Proteus mirabilis*. Thirty-five (70%) isolates produced MBL, while two (4%) produced both KPC and MBL carbapenemases. The remaining isolates (26%) produced neither KPC-type serine carbapenemases nor metallo-beta-lactamases. Around a quarter of carbapenem-resistant Enterobacteriaceae isolates tested in our hospital did not produce Class A or B carbapenemases or AmpC-type beta-lactamases and are presumed to have been carbapenem resistant with the help of Class D (OXA-type) carbapenemases, efflux pumps, porin loss or a combination of any of these factors.

Introduction

In the never ending war against bacteria, carbapenem resistance in both community-acquired and nosocomial infections, is one of our biggest challenges. Carbapenems are

active against both Gram-positive and Gram-negative bacteria, including anaerobes, with the exception of intracellular bacteria such as the Chlamydiae (Pitout and Laupland, 2008). Carbapenems have generally been considered to be resistant to older beta-lactamases but increasing resistance to this class of drugs is fast changing that.

Carbapenem-resistant Enterobacteriaceae (CRE) have been reported worldwide, and most strains have become resistant by acquiring carbapenemase genes (Queenan and Bush, 2007). These organisms have become a great concern because of the frequency with which they cause infections, the associated high mortality, and their potential to transmit carbapenem resistance via mobile genetic elements (Gupta *et al*., 2011). Apart from the expression of carbapenemases, resistance may be related to a decrease in outer-membrane permeability, over-expression of older β-lactamases with poor carbapenemase activity, or to expression of efflux pumps (Bush and Jacoby, 2011). Various carbapenemases have been reported in

Enterobacteriaceae, including *Klebsiella pneumoniae* carbapenemase (KPC; Ambler class A); Verona integron–encoded metallo-β-lactamase (VIM), imipenemase (IMP), New Delhi metallo-β-lactamase (NDM) (all Ambler class B); and oxacillinase-48 (OXA-48; Ambler class D) (Nordmann *et al*., 2011). In addition, carbapenemase producers are usually associated with other non–β-lactam resistance determinants, which give rise to multidrug- and pan drug-resistant isolates (Walsh and Tolemon, 2012).

Among the carbapenem resistance mechanisms, carbapenemase production is currently the most frequently encountered. While state-of-the-art techniques such as spectrometry and molecular assays are being developed, their availability and feasibility in the routine laboratory setting is questionable (Lee *et al*., 2013). In this context, the phenotypic tests have the advantage of being more practicable even in a simple laboratory setting. Different tests have been proposed to detect carbapenemases, using either phenotypic or genotypic techniques. So the present study was carried out to detect Class A Klebsiella pneumoniae carbapenemase (KPC) and similar serine carbapenemases, Class B metallo-beta-lactamases (MBL), and AmpC-type beta-lactamases in carbapenem-resistant Enterobacteriaceae isolates in a teaching hospital, using the proprietary KPC + MBL Detection Kit from Rosco Diagnostica, Denmark.

Material and Methods

The study includes all clinical specimens referred for bacteriological culture from outdoor (OPD) as well as indoor (IPD) patients of all age groups and both sexes from different wards of our hospital. The specimens comprised of urine, blood, sputum, pus, CSF, body fluids, swabs, endotracheal aspirate, tracheal tube aspirate, etc. Enterobacteriaceae isolates were identified by standard biochemical methods.

A total of 50 consecutive meropenem resistant clinical Enterobacteriaceae isolates received in the Department of Microbiology, Himalayan Institute of Medical Sciences, Jolly Grant Dehradun, India, from November 2013 to June 2014 were considered for this analysis. Meropenem resistance was determined using disk diffusion method as per the Clinical and Laboratory Standards Institute (CLSI, 2013) guidelines. These resistant isolates were further evaluated for detection of the KPC and MBL carbapenemases using the Rosco Diagnostica KPC+MBL detection kit.

An inoculum of the test strain was adjusted to a turbidity equivalent to 0.5 McFarland standard and grown on Mueller-Hinton agar plates as lawns. Four disks, namely meropenem (MRP), meropenem + boronic acid (MRPBO), meropenem + dipicolinic acid (MRPDP) and meropenem + cloxacillin (MRPCX) from Rosco Diagnostica kit were placed on each plate. The plates were then incubated at 37 ^{0}C for 24 hours and the results were interpreted according to manufacturer instruction. **(Table 1)**.

Result and Discussion

The 50 meropenem resistant isolates represented different bacterial populations The dominant species were *Klebsiella pneumoniae* with 22 (44%), *Escherichia coli* with 10 (20%) and *Enterobacter cloacae* with seven (14%) isolates. The remaining isolates included *Enterobacter aerogenes, Citrobacter freundii, Citrobacter koseri, Serratia marcescens, Proteus vulgaris and Proteus mirabilis*.

Of the 50 Enterobacteriaceae isolates tested by Rosco Diagnostica KPC+MBL kit ,35 strains were found to be MBL producers (**Figure 1**) i.e. the growth inhibitory zone diameter around the meropenem disc with dipicolinic acid had increased to >=5mm as compared to the growth inhibitory zone diameter seen around the disc containing meropenem alone. 2 isolates were found to coproduce both MBL and KPC, (**Figure 2**) as the growth inhibitory zone diameters seen around the meropenem disc with boronic acid and meropenem with dipicolinic acid had increased to >=5mm as compared to the growth inhibitory zone diameter seen around the disc containing meropenem alone .The rest of the 13 isolates were negative for both MBL and KPC productions, (**Figure 3**) as none of the discs showed any inhibitory activity.

Several phenotypic confirmation tests have been described for the detection of carbapenemase-producing Enterobacteriaceae. These include bioassays that detect the ability of these enzymes to hydrolyze the carbapenems (e.g., modified Hodge test [MHT]) and inhibitor-based methods using metal chelators for MBLs (e.g., MBL E-test), boronic acid for KPCs (Nordmann *et al*., 2012), and the commercial systems such as the Mast discs ID inhibitor combination disks and the Rosco Diagnostica Neo-Sensitabs KPC and MBL confirmation kit.

In our study we used Rosco Diagnostica KPC and MBL kit for the detection of different carbapenemases in Enterobacteriaceae. This inhibitor based assay was found to be useful in detecting carbapenemases in Enterobacteriaceae, with MBL being the most predominant mechanism of resistance. Similar findings have been reported in another study, wherein almost 100 per cent sensitivity and specificity have been reported on the use of inhibitor `assay for detection of MBL and KPC (Giske *et al*., 2013). In our study, 2 isolates showed the production of both KPC and MBL. Similar findings were also reported in the study done by Miriagoul *et al*., 2013. Inhibitor based assays for detection of MBL and KPC in formats such as epsilometer test, double disc approximation test and combination disc test are currently used as phenotypic confirmatory tests. Among these formats, the combination disc test is reported to perform superior, easier to carry out and interpret (Behera *et al*., 2008). The Rosco kit employs the combination principle using meropenem and three inhibitors for three types of carbapenemases. Boronic acid is a potent inhibitor of KPC and other types of serine carbapenemases except OXA.

Table.1 Interpretative criteria for Rosco KPC + MBL Detection Kit

Difference between Inhibition zone diameter around various discs			
MRPBO-MRP	MRPDP-MRP	MRPCX-MRP	Putative Carbapenem Resistant Mechanism
≥ 4 mm	< 5 mm	≥ 5 mm	Amp C Production
≥ 4 mm	< 5 mm	< 5 mm	KPC
< 5 mm	≥ 5 mm	< 5 mm	MBL

Key to abbreviations:

KPC- Klebsiella pneumoniae carbapenemases, **MBL-** Metallo-β-lactamases, **AmpC**-AmpC – type β- lactamases

MRP- Meropenem, **MRPBO-** Meropenem + Phenylboronic acid

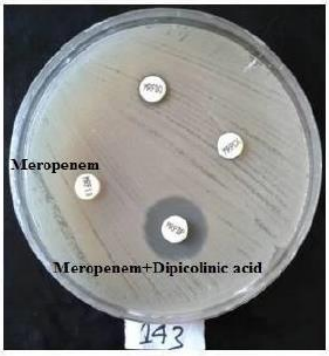
MRPDP- Meropenem + Dipicolinic acid, **MRPCX-** Meropenem + Cloxacillin

The following strains were used for quality control:
Klebsiella pneumoniae ATCC BAA-1705, KPC Positive

Klebsiella pneumoniae ATCC BAA-2146, MBL Positive

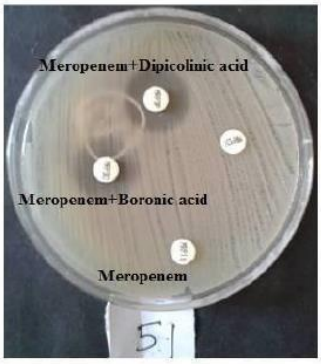
Klebsiella pneumoniae ATCC 700603, Negative control

Figure 1



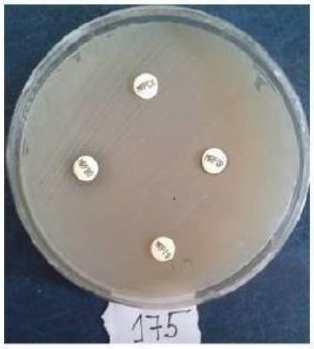
MBL Positive

Figure 2



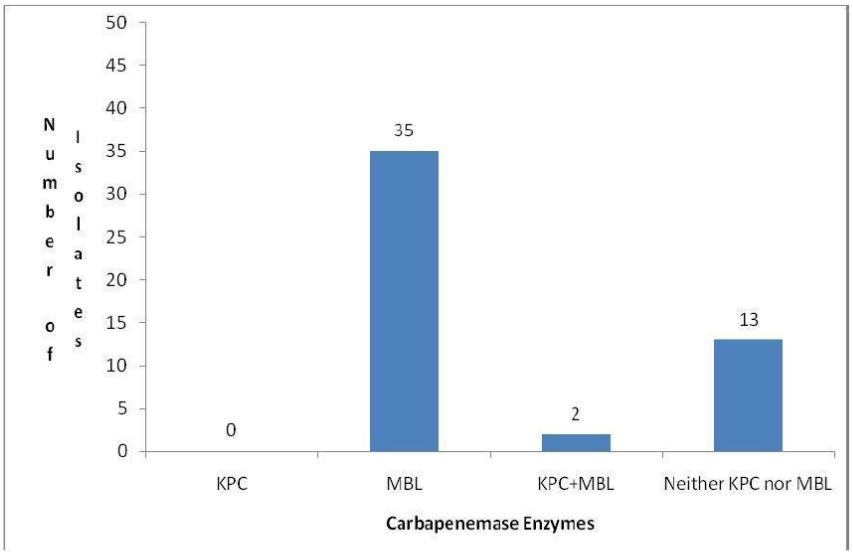
MBL+KPC Positive

Figure 3



Neither KPC nor MBL

Figure.4 Graph showing number of isolates with different carbapenemases



Studies have reported a very high sensitivity and specificity of this compound in detecting KPC (Nicola *et al*., 2012). EDTA and dipicolinate are potent chelators of divalent metal cations and are hence strong inhibitors of the zinc-dependent MBL. The AmpC-β-lactamase capable of hydrolysing carbapenems are rarely produced by Gram-negative bacteria including the Enterobacteriaceae (Dahyot *et al*., 2013). These carbapenemases are inhibited by both boronic acid and cloxacillin (Mirelis *et al*., 2006). The OXA types of carbapenemases are occasionally found in NFGNB and very rarely in the Enterobacteriaceae (Niranjan *et al*., 2013). Till date there are no compounds capable of inhibiting this class of carbapenemases. However, high level resistance to temocillin is an exclusive feature of OXA carbapenemases, not seen in other types of carbapenemases. Based on this property, the manufacturers have added a fifth tablet containing temocillin to the existing pack. This new pack is hence capable of identifying all types of carbapenemases at once. (Van Dijk *et al*., 2013). The clinical laboratory acts as an early warning system, alerting the medical community to new resistance mechanisms present in clinically important bacteria. We believe that the presence of carbapenemases among Enterobacteriaceae is an infection control emergency and that the detection of these bacteria in clinical laboratories is a critical step required for appropriate management of patients and infection prevention and control efforts. Clinical microbiology laboratories should be able to rapidly detect these enzymes among members of the Enterobacteriaceae, especially when these enzymes are first introduced into the local bacterial population. We recommend using such molecular tests for the optimal detection of these isolates and feel that initially it is important to know what type of carbapenemase is present. Unfortunately, these tests are expensive and often are

available only in large referral or research laboratories.

Our study showed the MBL as the major carbapenemase responsible for the carbapenem resistance in Enterobacteriaceae. The results of the present study correlate well with other recent studies, which suggest the Rosco kit as a reliable test in the detection of carbapenemases. The Rosco kit also has an added advantage that it can identify the class of carbapenemase produced and is also capable of differentiating the co-production of two different classes of carbapenemases by the same isolate. The use of Synergic assay allows to discriminate between different types of carbapenemases and can be suggested for routine diagnostic application because of its low cost, reliability, and very good discriminatory potential among different resistance mechanisms.

References

Behera, B., Mathur, P., Das, A., Kapil, A., Sharma, V. 2008. An evaluation of four different phenotypic techniques for detection of metallo-beta-lactamase producing *Pseudomonas aeruginosa*. *Indian. J. Med. Microbiol*., 26 : 233-7.

Bush, K., Jacoby, G. A. 2010. Updated functional classification of β-lactamases. *Antimicrob. Agents Chemother.,* 54(3): 969-76.

Clinical and Laboratory Standards Institute**.** 2012. Performance standards for antimicrobial susceptibility testing: twenty-second informational supplements. M100-S22. CLSI, Wayne, PA.

Dahyot, S., Broutin, I., Champs, C., Guillon, H., Mammeri, H. 2013. Contribution of asparagine 346 residue to the carbapenemase activity of CMY-2 β-lactamase. *FEMS Microbiol Lett*., 345: 147-53.

Giske, C. G., Gezelius, L., Samuelsen, O., Warner, M., Sundsfjord, A, Woodford, N. 2011. A sensitive and specific phenotypic assay for detection of metallo-β-lactamases and KPC in *Klebsiella pneumoniae* with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. *Clin. Microbiol. Infect.,* 17: 552-6.

Gupta, N, Limbago, B. M., Patel, J. B., Kallen, A. J. 2011. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin. Infect. Dis.,* 53(1): 60–7.

Lee, W., Chung, H. S., Lee, Y., Yong, D., Jeong, S. H., Lee, K, *et al*. 2013. Comparison of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry assaywith conventional methods for detection of IMP-6, VIM-2, NDM-1, SIM-1, KPC-1, OXA-23, and OXA-51carbapenemase-producing *Acinetobacter* spp., *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. *Diagn. Microbiol. Infect. Dis*., 77: 227-30.

Mirelis, B., Rivera, A., Miró, E., Mesa, R. J., Navarro, F., Coll, P. 2006. A simple phenotypic method for differentiation between acquired and chromosomal AmpC beta-lactamases in *Escherichia coli. Emer. Infec. Microbiol. Clin*., 24: 370-2.

Miriagou, V., Tzelepi, E., Kotsakis, S. D., Daikos, G. L., Bou Casals, J., Tzouvelekis, L. S. 2013. Combined disc methods for the detection of KPC- and/or VIM-positive *Klebsiella pneumoniae*: Improving reliability for

the double carbapenemase producers. *Clin. Microbiol. Infec.*, 19: E412-5.

Nicola, F. G., Nievas, J., Smayevsky, J. 2012. Evaluation of phenotypic methods for the detection of KPC carbapenemases in *Klebsiella pneumoniae*. *Rev Argent Microbiol.*, 44:290-302.

Niranjan, D. K., Singh, N. P., Manchanda, V., Rai, S., Kaur, I. R. 2013. Multiple Carbapene m hydrolyzing genes In clinical isolates of *Acinetobacter baumannii*. *Indian. J. Med. Microbiol.*, 31:237-41.

Nordmann, P., Gniafkowski, M., Giske, C. G., Poirel, L., Woodford, N. 2012. Identification and screening Of carbapenemase Producing Enterobacteriaceae. *Clin. Microbiol. Infect.*, 18:432– 438.

Nordmann, P., Naas, T., Poirel, L. 2011. Global spread of Carbapenemase producing *Enterobacteriaceae*. *Emerg. Infect. Dis.*, 17: 1791–1798.

Pitout, J. D., Laupland, K. B. 2008. Extended-spectrum–lactamase producing *Enterobacteriaceae*: An emerging public-health concern. *J. Lancet Infect. Dis.,* 8(1): 159-166.

Queenan, A.M., Bush, K. 2007. Carbapenemases: the versatile beta-lactamases. *Clin. Microbiol.* Rev., 20: 440–458.

Van Dijk, K., Voets, G. M., Scharringa, J., Voskuil, S., Fluit, A. C., Rottier, W. C., *et al.* 2013. A disc diffusion assay for detection of class A, B and OXA-48 carbapenemases in Enterobacteriaceae using phenyl boronic acid, dipicolinic acid and temocillin. *Clin. Microbiol. Infect.*

Walsh, T. R., Toleman, M. A. 2012. The emergence of pan-resistant gram-negative pathogens merits a rapid global political response. *J. Antimicrob. Chemothe.*, 67: 1–3.

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Comparative evaluation of rapid colorimetric methods for detecting carbapenemase enzymes in Gram-negative bacilli

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Abstract

Introduction: Most carbapenem-resistance in Gram-negative bacilli (GNB) is mediated by carbapenemase enzymes. It is therefore important to evaluate methods for their detection.

Aims: Comparing Carba NP with Blue Carba test in Enterobacteriaceae and *Pseudomonas aeruginosa*, and CarbAcineto NP with Blue Carba test in *Acinetobacter calcoaceticus baumannii* complex (ACBC). Also, assessing modifications of test protocols for possible improvement of performance.

Materials and Method: Total 184 carbapenem-resistant GNB were subjected to Carba NP, Blue Carba, and CarbAcineto NP tests as applicable. Variations were attempted in i) Purity of imipenem, and ii) carbapenemase induction by substrate exposure, to improve test performance. Isolates were also screened for carbapenemase genes by PCR.

Results: Carba NP and Blue Carba tests performed equally well with a sensitivity of 91.8% in Enterobacteriaceae and 61.2% in *P. aeruginosa*. CarbAcineto NP and Blue Carba tests were equivalent with 84.6% sensitivity in ACBC. Imipenem purity did not affect test performance. Carbapenemase induction by substrate exposure increased sensitivity by 6.25% only in Enterobacteriaceae. All strains carried the bla_{NDM-1} gene; some were positive for bla_{VIM} or bla_{OXA-48} genes too.

Conclusion: Our study is the first to demonstrate the equivalence of the Carba NP and Blue Carba tests in Enterobacteriaceae and *P. aeruginosa*. It is also the first to demonstrate the equivalence of the CarbAcineto NP and Blue Carba tests in ACBC. Minor modifications to test protocols did not improve performance, except for slightly improved sensitivity by enzyme induction in Enterobacteriaceae.

Keywords: Carbapenemase, Carba NP, CarbAcineto NP, Blue Carba

Introduction

Increasing resistance of Gram-negative bacilli to all available drugs, including carbapenems, is a major public health issue.[1,2] Carbapenem resistance is of especially great concern because these drugs have been the mainstay of empirical treatment of life-threatening infections for about two decades. Since most carbapenem resistance is mediated by carbapenemase enzymes, it is important to detect carbapenemases in a timely and economic manner to guide treatment and control.[3, 4]

Both phenotypic and genotypic methods exist for detecting carbapenemases. Phenotypic methods are popular, not only because they are rapid, user-friendly and relatively inexpensive, but also because they can detect novel enzymes that cannot be picked up by genotypic methods. Genotypic methods, on the other hand, are more sensitive than their phenotypic counterparts.[5,6]

Nordmann et al. described the Carba NP test (CNP) in 2012. CNP is a rapid assay based on the hydrolysis of the β-lactam ring of imipenem in a weak buffer, with consequent acidification of the solution detected by the pH indicator, phenol red.[7]

More recently, Pires et al. proposed the Blue Carba test based on the same principle. In the Blue-Carba test variant, bromothymol blue is used as the indicator instead of phenol red because the pH range (6.0 to 7.6) of the former overlaps the optimum pH (pH 6.8) of most β-lactamases. The increased sensitivity obtained thereby allows the test to be done on intact cells picked up from a colony without an intermediate step of cell lysis.[8]

With some changes, Nordmann et al. proposed one more method, i.e., the CarbAcineto NP for the detection of carbapenemases in *Acinetobacter* spp. In this modification, the buffer is replaced by sodium chloride solution.[9]

All these tests are highly sensitive and their results can be read in two hours, making them suitable for use in most laboratories. Therefore, the present study was planned to compare the performance of Carba NP, CarbAcineto NP and Blue Carba tests for detecting carbapenemase production in Gram-negative bacilli. In addition, certain test parameters were modified in an attempt to improve test performance. PCR was carried out to detect common carbapenemase genes.

Materials and Method

The study included non-replicate strains of Gram-negative bacilli isolated from clinical specimens of patients admitted to our tertiary-care hospital between January and June, 2015. Gram-negative bacilli were identified by standard biochemical methods; strains that could not be identified by manual testing were identified with the automated VITEK 2 system. Antimicrobial susceptibility testing was performed by the Kirby-Bauer

method and results interpreted according to CLSI guidelines.[10]Mueller Hinton Agar and antimicrobial discs were procured from HiMedia®; Mumbai, India.

Isolates resistant to carbapenem drugs on disc diffusion testing were tested with Etest® strips (bioMérieux) for confirmation.

Carba NP, CarbAcineto NP and Blue Carba tests were performed after growing strains overnight on Mueller-Hinton II agar (BBL, France) with imipenem Neo-Sensitabs™ discs (Rosco Diagnostica, Denmark).

The performance of the Carba NP test was compared to that of Blue Carba in Enterobacteriaceae and *P. aeruginosa*. The performance of the CarbAcineto NP test was compared to that of Blue Carba in *Acinetobacter calcoaceticus baumannii* complex (ACBC). All tests were performed as described.[7,8,9]

In addition, 'in-house' variations of all these tests were attempted, as follows, in an attempt to reduce costs or improve sensitivity.

1. Imipenem of two different grades were used: more expensive, analytical grade chemical from Sigma-Aldrich™, and less expensive, pharmaceutical grade imipenem-cilastatin from Lupinem®, India.
2. Bacterial biomass for the tests was taken from two different sites on the same plate: one near the carbapenem disc and other away from it.

Escherichia coli ATCC®25922™ (pan-sensitive), *Pseudomonas aeruginosa* ATCC®27853™ (pan-sensitive), *Klebsiella pneumoniae* ATCC®BAA-1705™ (KPC Positive), and *Pseudomonas aeruginosa* ATCC®10145™ (MBL Positive) were used for quality control.

Results

Our study showed a very high rate of carbapenem resistance (184/1544; 11.9%) in Gram-negative bacilli isolated at our tertiary-care hospital. Resistant isolates included *Acinetobacter calcoaceticus baumannii* complex (56.5%; 104/184), *P. aeruginosa* (31/184; 16.8%), and various species of Enterobacteriaceae (49/184; 26.6%). Among the latter, *Klebsiella pneumoniae* and *Escherichia coli* were the predominant species. Most resistant isolates had carbapenem MICs ≥32 µg/ml.

All carbapenem-resistant isolates in our study were positive for the bla_{NDM-1} gene. In addition, some strains carried the bla_{VIM} and bla_{OXA-48} genes.

The Carba NP and Blue Carba tests had an identical sensitivity of 91.8% (45/49) for Enterobacteriaceae and 61.2% (19/31) for *P. aeruginosa* isolates for detecting carbapenemase enzymes.

The CarbAcineto NP and Blue Carba tests had an identical sensitivity of 84.6% (88/104) for detecting carbapenemase enzymes in ACBC strains; positive results were obtained in the same strains in all cases.

The purity of imipenem, i.e., analytical or pharmaceutical grade, made no difference to results.

The site from where bacterial biomass was collected relative to the position of the imipenem sensitivity testing disc on the culture plate made no difference to the results in case of *P. aeruginosa* and ACBC. In case of Enterobacteriaceae, three (6.25%) strains gave a positive result only when bacterial biomass was taken from near the imipenem sensitivity testing disc, suggesting that carbapenemase production was induced by imipenem in these strains.

Photographs of the results of various tests and their variations are shown in Fig. 1.

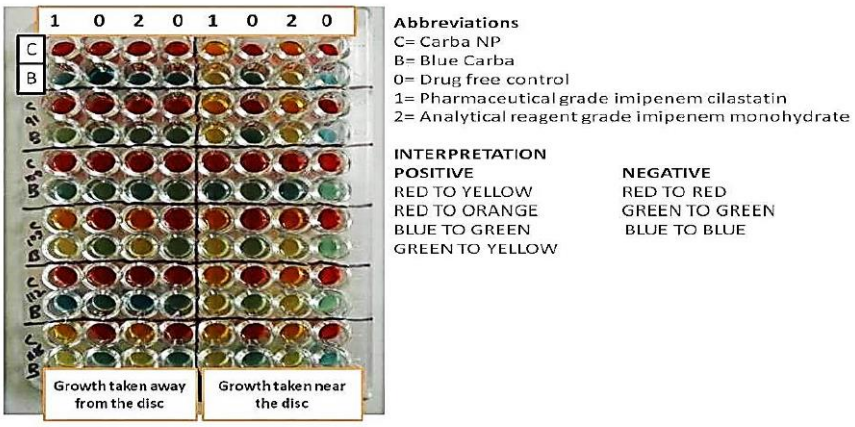

Fig. 1: Various tests with their variations

Discussion

Several studies have evaluated the Carba NP, CarbAcineto NP, Blue Carba tests in the past[11-14] but ours is the first to compare the Carba NP test directly with the Blue Carba test in Enterobacteriaceae and *P. aeruginosa*, and the CarbAcineto NP test with the Blue Carba test in *Acinetobacter calcoaceticus baumannii* complex. It is noteworthy that in our hands the Blue Carba test did not perform any better than the older tests, despite claims of its being an improvement over the Carba NP and CarbAcineto NP tests.[8]

The sensitivity of the Carba NP and Blue Carba tests were 91.8% (45/49) in Enterobacteriaceae. However, these tests were significantly less sensitive (61.2%; 19/31) in *P. aeruginosa*. This is similar to the sensitivity rate (37%) reported by Pragasam *et al*,[15] and is also supported by CLSI guidelines (M100-S26) which mention a lower sensitivity of the Carba NP test in *P. aeruginosa*.

The CarbAcineto NP and Blue Carba tests had an identical sensitivity of 84.6% (88/104) for detecting carbapenemase enzymes in ACBC strains, yielding positive results in the same strains in all cases. This is similar to the findings of Vijayakumar *et al* who reported 91% positivity by the CarbAcineto NP test.[16]

Substituting analytical grade imipenem with pharmaceutical grade imipenem-cilastatin made no difference to test performance, provided the concentration of imipenem was doubled when using imipenem-cilastatin. Similar results have reported by Hartl *et al* too.[17]

Harvesting bacterial mass adjacent to an imipenem disk improved the performance of the Carba NP and Blue Carba tests by 6.25% in Enterobacteriaceae. Ours is the first study to report an improvement of test performance consequent to enzyme induction by substrate exposure. Interestingly, the same effect was not seen in ACBC or *P. aeruginosa*.

All carbapenem-resistant isolates in our study carried the bla_{NDM-1} gene. A similar situation was reported from a hospital in Mumbai in 2010 by Deshpande *et al*.[18] In addition, some strains carried the bla_{VIM} and bla_{OXA-48} genes too.

Conclusion

Carba NP and Blue Carba tests performed equally well in our hands for detecting carbapenemases in Enterobacteriaceae and *P. aeruginosa*. Similarly, no difference was found in the performance of the CarbAcineto NP and Blue Carba tests for detecting carbapenemases in *Acinetobacter* spp. All tests performed equally well with reagent grade imipenem or pharmaceutical grade imipenem-cilastatin, provided the quantity of imipenem was doubled in the latter. Carbapenemase induction through proximity to an imipenem disc improved test sensitivity marginally in Enterobacteriaceae, but not in *P. aeruginosa* or Acinetobacter.

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References

1. Mesaros N, Nordmann P, Plesiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, Van Laethem Y, Jacobs F, Lebecque P, Malfroot A, Tulkens PM, Van Bambeke F.. *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. Clin. Microbiol. Infect. (2007) 13, 560-78.
2. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm. Trends Mol. Med. (2012) 18, 263-72.
3. Stuart JC, Hall LV, M. Guideline for phenotypic screening and confirmation of carbapenemases in Enterobacteriaceae. International Journal of Antimicrobial Agents (2010) 36,205–210.
4. Kaase M, Szabados F, Wassill L. Detection of Carbapenemases in Enterobacteriaceae by a Commercial Multiplex PCR. Journal of Clinical Microbiology (2012) 50, 3115–3118.
5. Thomson KS. Extended-spectrum-beta-lactamase, AmpC, and Carbapenemase issues. Journal of Clinical Microbiology. (2010) 48,1019–10125.
6. Bush K, Pannell M, Lock JL, Queenan AM, Jorgensen JH, Lee RM, Lewis JS, Jarrett D.. Detection systems for carbapenemase gene identification should include the SME serine carbapenemase. International Journal of Antimicrobial Agents. (2013) 41, 1–4.
7. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. Emerging Infectious Diseases. (2012) 18, 1503–1507.
8. Pires J, Novais A, Peixe L. Blue-Carba, an Easy Biochemical Test for Detection of Diverse Carbapenemase Producers Directly from Bacterial Cultures. Journal of Clinical Microbiology (2013) 51(12), 4281–4283.
9. Dortet L, Poirel L, Errera C, Nordmann P. CarbAcineto NP Test for Rapid Detection of Carbapenemase-Producing *Acinetobacter* spp. Journal of Clinical Microbiology. (2014) 52(7), 2359–2364.
10. Clinical and Laboratory Standard Institute. Performance standard for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement M100-S25 Wayne, PA: CLSI: 2015.
11. Dortet L, Poirel L, Nordmann P. Further proofs of concept for the Carba NP test. Antimicrobial Agents Chemotherapy (2014) 58, 1269.
12. Dortet L, Brechard L, Poirel L, Nordmann P. Impact of the isolation medium for detection of carbapenemase-producing *Enterobacteriaceae* using an updated version of the Carba NP test. Journal of Medical Microbiology (2014) 63, 772-776.
13. Vasoo S, Cunningham SA, Kohner PC, Simner PJ, Mandrekar JN, Lolans K, Hayden MK, Patel R. Comparison of a novel, rapid chromogenic biochemical assay, the Carba NP test, with the modified Hodge test for detection of carbapenemase-producing Gram-negative bacilli. Journal of Clinical Microbiology (2013) 51, 3097–3101.
14. Rao A, Indumathi VA. Detection of Carbapenem Resistant Enterobacteriaceae from Clinical Isolates. International Journal of Current Microbiology and Applied Sciences. (2016) 5(5), 864-869.

15. Pragasam AK, Vijayakumar S, Bakthavatchalam YD, Kapil A, Das BK, Ray P, et al. Molecular characterisation of antimicrobial resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* during 2014 and 2015, collected across India. Indian Journal of Medical Microbiology (2016) 34, 433-41.
16. Vijayakumar S, Gopi R, Gunasekaran P, Bharathy M, Walia K, Anandan S, Veeraraghavan B.. Molecular Characterization of Invasive Carbapenem- Resistant *Acinetobacter baumannii* from a Tertiary Care Hospital in South. Infectious Disease and Therapy (2016) 5, 379–387.
17. Hartl R, Widhalm S, Kerschner H. Cilastatin does not affect Carba NP test performance for detection of carbapenemase production in Enterobacteriaceae. Wiener Klinische Wochenschrift (2017) 129(1-2), 29-32.
18. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, Soman R. New Delhi Metallo-beta lactamase (NDM-1) in Enterobacteriaceae: treatment options with carbapenems compromised. J Assoc Physicians India.(2010) 58, 147-9.

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Universal Presence of blaNDM-1 Gene in Carbapenem-Resistant Gram-Negative Bacilli in an Indian Hospital in 2015

BISWAROOP CHATTERJEE[1], NARESH KHANDURI[2], BARNALI KAKATI[3], AARTI KOTWAL[4]

Keywords: Enterobacteriaceae, Minimum inhibitory concentration, Polymerase chain reaction

Dear Editor,

Carbapenem-resistant Gram-Negative Bacilli (GNB) which is simultaneously resistant to most other antimicrobials is now found in many hospitals worldwide [1]. Resistant strains are associated with high mortality; therefore, it is important to investigate resistance mechanisms to guide efforts to combat them.

The study started with all (1544) GNB strains isolated from routine clinical specimens received at the diagnostic laboratory of our tertiary-care hospital during the period January 2015 to June 2015. Out of these strains, 194 were found to be carbapenem-resistant by the Kirby-Bauer disc diffusion method, interpreted according to Clinical Laboratory Standard Institute (CLSI) [2] guidelines, were included in the study. Subsequently, six isolates of *Elizabethkingia meningoseptica* and four of *Stenotrophomonas maltophilia* were excluded because of intrinsic carbapenem-resistance. Carbapenem Minimum Inhibitory Concentrations (MIC) of the remaining (184) strains were determined with E-test [3] strips (bioMérieux, India) on Muller Hinton II Agar (Becton Dickinson, USA); MIC values were interpreted according to CLSI guidelines. Mean carbapenem MICs of resistant isolates were above 25 µg/ml for all organisms/carbapenem combinations studied. Isolates were identified with standard biochemical methods [4], supplemented with Vitek 2 GNID panels if needed. Resistant strains were comprised of 104 isolates of *Acinetobacter Calcoaceticus Baumannii* Complex (ACBC), 49 of family Enterobacteriaceae, and 31 of *Pseudomonas aeruginosa*. Among Carbapenem-Resistant Enterobacteriaceae (CRE), *Klebsiella pneumoniae* (17) and *Escherichia coli* (15) were the most common species, followed by *Enterobacter cloacae* (7), *Enterobacter aerogenes* (5), *Citrobacter freundii* (4), and *Citrobacter koseri* (1).

PCR was performed for *blaNDM-1*, *blaVIM*, *blaKPC* and *blaOXA-48* carbapenemase genes with positive and negative controls in each run [5-7]. Phenotypic tests for carbapenemases were used too; these included Modified Hodge Test (MHT), Carba NP Test (CNPT), Blue Carba test (BCT), and Carba Acineto NP test (CANPT). MHT and CNPT were performed according to CLSI protocols [2], while BCT and CANPT were performed according to protocols in publications reporting these tests for the first time [8,9]. No phenotypic assay for carbapenemase detection had sensitivity above 90% in our hands when compared with PCR.

All GNB isolates with acquired carbapenem resistance carried the *blaNDM-1* gene [Table/Fig-1]. In addition, the *blaVIM* gene was detected in 24 isolates, which included *P. aeruginosa* (20), *Acinetobacter calcoaceticus baumannii complex* (03) and *Enterobacter cloacae* (01). The *blaOXA-48* was detected only in *K. pneumoniae* (08). No isolate carried the *blaKPC* gene.

Studies on NDM-1 in Southern Asia, starting with the seminal article by Kumarasamy KK et al. in 2010, are too numerous to quote [10].

		Acinetobacter	*P. aeruginosa*	*Enterobacteriaceae*
Total strains 184				
Number of strains carrying the gene concerned	NDM-1	104	31	49
	VIM	03	20	01
	OXA-48	0	0	08
	KPC	0	0	0

[Table/Fig-1]: Carbapenemase genes in carbapenem-resistant Gram-negative bacilli. (Total strains 184)

The prevalence of *blaNDM-1* gene in India has increased steadily since then, and a PubMed search revealed an article from 2012 reporting its presence in all (17) carbapenem-resistant isolates of *K. pneumoniae* in Guwahati, Assam, India [11]. Another study from 2014 reported the presence of *blaNDM-1* gene in all [12] carbapenem-resistant isolates in Sharjah, UAE, where many patients travel frequently to Southern Asia [13]. However, ours is the first to report the universal presence of the *blaNDM-1* gene in such a large number (184) of carbapenem-resistant isolates. Ours is also the first to report the high (12.56%) incidence of carbapenem-resistance in clinical isolates of GNB, and the presence of *blaNDM-1*, from our mountainous state in Northern India.

The universal presence of blaNDM-1 in our carbapenem-resistant isolates, along with similar or identical findings in other places in Asia, is worrisome because Ambler Class B metallo-beta-lactamases are not inhibited by the newly developed beta-lactamase inhibitors, avibactam and relebactam, which target serine carbapenemases of Ambler Class A and C only. This emphasizes the need to develop inhibitors of Ambler Class B carbapenemases. Fortunately, cyclobutanone and bisthiazolidine derivatives have displayed promising activity against metallo-beta-lactamases, and it is hoped that structural modifications will improve their activity to clinically significant levels in the near future [12,14].

To conclude, it is important to monitor the nature of carbapenemases to provide impetus to the development of newer inhibitors, and also guide their subsequent use, especially on an empiric basis. Since it is neither feasible nor economical to do this on all carbapenem-resistant isolates, nationally coordinated surveys must be done periodically with significant numbers of geographically representative isolates to maintain an up-to-date picture of resistance mechanisms in different parts of the country.

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REFERENCES

[1] Walsh TR. Emerging carbapenemases: A global perspective. Int J Antimicrob Agents. 2010;36(13):S8-S13.
[2] Clinical and Laboratory Standard Institute. Performance standard for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement M100-S25 Wayne, PA:CLSI: 2015.
[3] Steward CD, Mohammed JM, Swenson JM, Stocker SA, Williams PP, Gaynes RP, et al. Antimicrobial Susceptibility Testing of Carbapenems: Multicenter Validity Testing and Accuracy Levels of Five Antimicrobial Test Methods for Detecting Resistance in Enterobacteriaceae and Pseudomonas aeruginosa Isolates. J Clin Microbiol. 2003;41(1):351-58.
[4] Lennette EH, Balows A, Hausler WJ, Shadomy HJ. Manual of Clinical Microbiology 4th Edn. Washington, D.C American Society for Microbiology, 1985.
[5] Monteiro J, Santos AF, Asensi MD, Peirano G, Gales AC. First report of KPC-2 producing *Klebsiella pneumoniae* strains in Brazil. Antimicrob Agents Chemother. 2009;53(1):333–34.
[6] Monteiro J, Widen RH, Pignatari ACC, Kubasek C, Silbert S. Rapid detection of carbapenemase genes by multiplex real-time PCR. J Antimicrob Chemother. 2012;67(4):906–09.
[7] Mendes RE, Kiyota KA, Monteiro J, Castanheira M, Andrade SS, Gales AC, et al. Rapid detection and identification of metallo-β-lactamase-encoding genes by multiplex real-time PCR assay and melt curve analysis. J Clin Microbiol. 2007;45(2):544–47.
[8] Pires J, Novais A, Peixe L. Blue-Carba, an Easy Biochemical Test for Detection of Diverse Carbapenemase Producers Directly from Bacterial Cultures. J Clin Microbiol. 2013;51(12):4281–83.
[9] Dortet L, Poirel L, Errera C, Nordmann P. CarbAcineto NP test for rapid detection of carbapenemase-producing Acinetobacter spp. J Clin Microbiol. 2014;52(7):2359–64.
[10] Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10(9):597-602.
[11] Bora A, Ahmed G. Detection of NDM-1 in clinical isolates of *Klebsiella pneumoniae* from North-East India. J Clin Diagn Res. 2012;6(5):794-800.
[12] Drawza SM, Papp-Wallace KM, Bonomo RA. New β-Lactamase Inhibitors: a Therapeutic Renaissance in an MDR World. Antimicrob Agents Chemother. 2014;58(4):1835-46.
[13] Dash N, Panigrahi D, Zarouni MA, Darwish D, Ghazawi A, Sonnevend A, et al. High incidence of New Delhi metallo-beta-lactamase-producing *Klebsiella pneumoniae* isolates in Sharjah, United Arab Emirates. Microb Drug Resist. 2014;20(1):52-56.
[14] Gonzalez MM, Kosmopoulou M, Mojica MF, Castillo V, Hinchliffe P, Pettinati I, et al. Bisthiazolidinones: a substrate-mimicking scaffold as an inhibitor of the NDM-1 carbapenemase. ACS Infect Dis. 2015;1(11):544-54.

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