DOI: 10.7860/JCDR/2016/16417.8152

Original Article

Microbiology Section

A Pilot Study on Carbapenemase Detection: Do We See the Same Level of Agreement as with the CLSI Observations

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ABSTRACT

Introduction: Rapid identification of carbapenemase producing organisms is of great importance for timely detection, treatment and implementation of control measures to prevent the spread. The Modified Hodge Test (MHT) and Carba NP test is recommended by CLSI for the detection of carbapenemases in *Enterobacteriaceae*. However, MHT may give false positive results or fail to detect metallo β-lactamases (MBLs). In the US, MHT is the most widely used test for detection of carbapenemases and has been found to have a sensitivity and specificity of >90% for bla_{KPC} producers. However, in India, the prevalence of bla_{NDM} is higher than bla_{KPC} producers.

Aim: To evaluate the usefulness of CarbaNP in an Indian setting.

Materials and Methods: A total of 260 isolates of carbapenem resistant *E.coli (n=57), Klebsiella* spp. *(n=85), Pseudomonas aeruginosa (n=60)*, and *Acinetobacter baumannii (58)* isolated from clinical specimens between 2012-2014 at the Christian Medical College, Vellore were included in the study. All the carbapenem resistant isolates were subjected to CarbaNP, MHT and multiplex PCR for detection of carbapenemase genes.

Results: CarbaNP was found to be positive in 88% *(n=50/57)*, 81% *(n=69/51)*, 38% *(n=23/60)* and 81% *(n=47/58)* for *E.coli, Klebsiella* spp., *P. aeruginosa*, and *A. baumannii* respectively. While in MHT it showed, 89% *(n=51/57)* and 81 % *(n=69/85)* for *E.coli and Klebsiella* spp. respectively. In *P.aeruginosa,* synergy testing of imipenem plus cloxacillin showed that, 65% of CarbaNP negatives were *ampC* producers. Overall, the sensitivity and specificity of CarbaNP was found to be 94% and 100 for bla_{NDM}; 77% and 100 % for $bla_{OXA\text{-}48\ like}$ producers and 81% and 100% for CarbAcinetoNP respectively.

Conclusion: This observation was more than what was reported in CLSI guidelines. Therefore, it is advisable to evaluate an assay for better laboratory diagnosis at respective regions.

Keywords: CarbaNP, MHT, Metallo beta lactamases, Class D Oxacillinases

INTRODUCTION

Carbapenems are considered to be the drugs of last resort for treating multi drug resistant pathogens due to their excellent clinical efficacy and safety profiles. Resistance to carbapenem groups of drugs is on the rise. It is especially of concern in the nosocomial pathogens which are multidrug resistant. The most common mechanism of carbapenem resistance is the production of carbapenemase enzymes. These enzymes are carried on mobile plasmids and have a greater propensity to cause outbreaks [1]. Carbapenemases are classified based on their functional and molecular properties. Molecular classes A and D are the β-lactamases having serine at their active site, whereas molecular class B β-lactamases are all metallo-enzymes with zinc at their active site. Carbapenemases belonging to class A of molecular classification include bla_{KPC}, bla_{GES}, bla_{IMI}, bla_{SME} and bla_{SFC}

Ambler class B, carbapenemases which were widely found in *Pseudomonas* and other non-fermenting Gram negative bacilli are now being increasingly identified in *Enterobacteriaceae* and are inhibited by EDTA [2]. Various families of MβLs are identified in different parts of the world namely bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{GIM} and bla_{SIM}. Of these, members of VIM and IMP family have a worldwide distribution. Recent identification of New Delhi metallo-β-lactamase-1 ($bla_{NDM\text{-}1}$) producers, originally in the United Kingdom, India, and Pakistan and now reported worldwide which is worrisome. Importantly, bla_{NDM} is now the most commonly isolated enzyme from the Indian subcontinent and is rapidly spreading worldwide [3]. Carbapenemases of the oxacillinase-48 type have been identified mostly in Mediterranean and European countries and in India. This group of β-lactamases is capable of hydrolyzing penicillins, cloxacillin, oxacillin. Their prevalence is on a rise and recent studies found that these were the predominant carbapenemases in countries like France and Belgium [4,5].

Carbapenemases are a versatile group of β-lactamases that are characterized by their resistance to virtually all β-lactam antibiotics, including the cephalosporins and carbapenems. However, the hydrolysis profile varies for each group of carbapenemases [6], wherein metallo β-lactamases (MBLs) does not hydrolyze aztreonam. Till date, many numbers of different carbapenemases have been identified and within each carbapenemases, many variants have been found (Laheystudies.org). In India, the most common carbapenemase are bla_{NDM}, bla_{VIM} and $bla_{OXA\text{-}48\ like}$ enzymes [7]. Rapid detection of carbapenemases using tests with a short turnaround time is essential to ensure early detection and timely implementation of control measures in hospitals. Preliminary screening is with the Disk Diffusion (DD) testing of imipenem or meropenem which is done on routine basis in most of the laboratories. For confirmatory testing, the Modified Hodge test (MHT) is recommended by CLSI for the detection of carbapenemases in *Enterobacteriaceae* [8]. However, MHT may give false positive results [9] or fail to detect MBLs and can detect up to11% of the isolates [8]. In the US, MHT is the most widely used test for detection of carbapenemases and has been found to have a sensitivity and specificity of >90% for bla_{KPC} producers [8]. However, in India, the prevalence of bla_{NDM} is higher than that of bla_{KPC} producers [10].

In contrast, many groups have reported higher sensitivity in detecting MBLs above 11% as mentioned by CLSI. Additionally, a study has shown it may be possible to improve the sensitivity of MHT in detecting the MBL producers by adding zinc to the test medium as well [11]. Nordmann and Poirel described Carba NP for the detection of carbapenemase producing clinical isolates of *Enterobacteriaceae* and *Pseudomonas* spp. [12,13]; and CarbAcineto NP for *Acinetobacter* spp. [14]. Recently, Carba NP has been introduced as a confirmatory test for carbapenemase producers by CLSI M100-S25 [15].

As the carbapenem resistance rates are high, rapid detection is very important for guiding appropriate antimicrobial therapy and also to implement appropriate control measures. Henceforth, we undertook this pilot study to evaluate MHT and CarbaNP test for timely detection of carbapenemase producers from pathogens isolated from the clinical specimens at a tertiary care centre in South India.

MATERIALS AND METHODS

Phenotypic Characterization

Bacterial isolates: A total of 260 isolates resistant to imipenem and meropenem isolated from blood and respiratory specimens were included in this study prospectively from all age groups. Of these 57, 85, 60 and 58 were carbapenem resistant *E.coli*, *Klebsiella* spp., *Pseudomonas aeruginosa* and *Acinetobacter baumannii* respectively. All the study isolates were identified up to species level as per standard protocols which includes, mannitol motility, triple sugar iron, citrate utilization and indole production test for *Enterobacteriaceae*; Oxidase, catalase, nitrate reduction, sugar fermentation such as lysine, ornithine and arginine tests for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [16]. They were sourced from various clinical specimens collected between 2012 and 2014 at Christian Medical College, Vellore, South India. The screening for the carbapenem resistance for the study isolates were carried out using Kirby Bauer disc diffusion for imipenem (10µg) and meropenem (10µg) along with the routinely tested antimicrobial agents such as cephalosporins (cefotaxime, ceftazidime); β-lactam/β-lactamase inhibitors (Piperacillin/tazo bactam, cefoperazone/sulbactam); carbapenems (imipenem, meropenem); fluoroquinolones (ciprofloxacin, levofloxacin); PB300 units and tigecycline (For *Klebsiella* spp. and *Acinetobacter spp.)* and interpreted according to CLSI guidelines M100-S22, M100-S23, M100-S24.

Modified Hodge Test: MHT was performed only for *E.coli* and *Klebsiella spp.* as per CLSI recommendations M100-S21 [8]. Lawn culture of 1 in 10 diluted, 0.5 McFarland turbidity of susceptible ATCC 25922 *E.coli* was made and ertapenem disc of 10 µg/ml was placed in the centre. Test isolate and controls were streaked onto the lawn culture from centre to periphery. Following 24 hours incubation, presence of clover leaf like indentation was taken as positive for the carbapenemase production and absence of indentation was taken as negative. *K.pneumoniae* ATCC BAA 1705 and *K. pneumoniae* ATCC BAA 1706 were used as positive and negative controls for all the assays respectively. Presence of clover leaf like indentation along the streak line of the test isolate indicating the growth of the carbapenem susceptible strain (*E.coli*ATCC 25922) was taken as a positive for carbapenemase production. While, absence of indentation was taken as a negative result for carbapenemase production.

Carba NP test: For all carbapenem resistant *E.coli*, *Klebsiella* spp. and *P. aeruginosa,* Carba NP test was done and a modified protocol of CarbAcineto NP was used for *A. baumannii* as recommended by Nordmann and Poirel [6-8]. Since Class D Oxacillinases are more prevalent in *Acinetobacter* spp., a modified protocol using 5 M NaCl was used instead of B-PER II lysis buffer, to avoid any buffer effect, as Oxa enzymes possess weak carbapenemase activity. All the study isolates subjected to CarbaNP and CarbAcineto NP were grown on Mueller Hinton agar plates for 24 hours and the isolated colonies were used for testing. *K. pneumoniae* ATCC BAA 1705 and *K. pneumoniae* ATCC BAA 1706 were used as positive and negative controls in all the assays, respectively. CarbaNP test was performed in two 1.5ml Eppendorf tubes labeled as control and test. 0.1 ml of B-PER II Lysis buffer (Thermo Scientific Cat.no. 78260) was taken in both the tubes. 10 µl loopfull of colonies to be tested was inoculated in both the tubes and vortexed briefly. Following that, 0.1ml of phenol red solution with 6µg/ml imipenem was added in the tube labeled as test and 0.1 ml of phenol red solution without imipenem was added in the tube labeled as control. The tubes were incubated at 37°C for upto two hours. Every 15 minutes once, the tubes were checked for the colour change. Change of the phenol red indicator from red to yellow was taken as a positive for carbapenemase producers. Absence of colour change from red to yellow was taken as negative result. The observation of colour change was taken by two independent readers. Imipenem+cloxacillin test: Combination disc test of imipenem and imipenem plus cloxacillin was used to demonstrate the synergy of cloxacillin with imipenem in order to differentiate between carbapenemase and non-carbapenemase producers (only for *P.aeruginosa*) [17]. Cloxacillin of 4000µg/ml was used alone and in combination with imipenem (10 µg/ml). Zone diameter of >5mm difference between imipenem alone and in combination of imipenem plus cloxacillin was taken as a positive result for hyper AmpC producers.

Molecular Characterization

Multiplex PCR for Detection of Carbapenemase genes

All the test isolates were grown on blood agar overnight and whole genomic DNA was extracted by boiling lysis method [18]. Conventional multiplex PCR was done for the detection of carbapenemase genes such as bla_{SPM}, bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC} and $bla_{OXA\text{-}48\ like}$. In addition, $bla_{OXA\text{-}51}$, $bla_{OXA\text{-}23}$ and $bla_{OXA\text{-}24}$ genes were screened for *A. baumannii* isolates. Further, presence of $bla_{OXA\text{-}51}$ was carried out to confirm that all studied isolates are *A.baumannii* [19-25]. The amplicons were visualized in 2% agarose gel with staining of ethidium bromide. Known positive controls for appropriate genes were used (Courtesy: IHMA, Inc., USA). Primers and cycling conditions are mentioned in [Table/Fig-1]. Furthermore, a total of 30 carbapenem susceptible isolates of *E.coli* and 30 carbapenem susceptible *Klebsiella* spp. were included in the assay as a comparator.

Genes	Primers	Product Size (bp)	Annealing temp (°C)	References
bla_{IMP}	GGAATAGAGTGGCTTAAYTCTC	232	59	[11]
	GGTTTAAYAAAACAACCACC			
bla_{VIM}	GATGGTGTTTGGTCGCATA	390	59	[12]
	CGAATGCGCAGCACCAG			
$bla_{OXA\text{-}48\ like}$	TATATTGCATTAAGCAAGGG	800	59	[13]
	CACACAAATACGCGCTAACC			
bla_{NDM}	CACCTCATGTTTGAATTCGCC	984	59	[14]
	CTCTGTCACATC GAAATCGC			
bla_{KPC}	TGTCACTGTATCGCCCGTC	1011	59	[15]
	CTGAGTGCTCTACAGAAAACC			
bla_{SPM}	AAAATCTGGGTACGCAAACG	271	59	[16]
	ACATTATCCGCTGGAACAGG			
$bla_{OXA\text{-}51}$	TAATGCTTTGATCGGCCTTG	353	57	[17]
	TGGATTGCACTTCATCTTGG			
$bla_{OXA\text{-}23\ like}$	GATCGGATTGGAGAACCAGA	501	52	
	ATTTCTGACCGCATTTCCAT			
$bla_{OXA\text{-}24/40\ like}$	GGTTAGTTGGCCCCCTTAAA	246	52	
	AGTTGAGCGAAAAGGGGATT			

[Table/Fig-1]: Primers used for the carbapenemase gene identification.

Statistical Method

Statistical analysis of the categorical data was done using SPSS Version 16.0. The sensitivity and specificity of CarbaNP test were calculated and compared with PCR results as a gold standard, for the individual carbapenemase encoding genes respectively.

RESULTS

CarbaNP was found to be positive in 88% (*n=50*/57) of *E.coli,* 81% (*n*=69/85) of *Klebsiella* spp., 38% (*n*=23/60) of *P. aeruginosa* and

	Carba NP	MHT	bla_{NDM}	$bla_{Oxa48\ like}$	bla_{NDM}+bla_{Oxa-48} * bla_{NDM}+bla_{VIM} †	No carbapenemase detected	Remarks
Total no tested: *Klebsiella pneumoniae (n=85)*							
Klebsiella spp. (n=68)	+	+	18	35	13* ; 1†	1‡	Among $bla_{oxa-48\ like}$ producer, 36 out of 47 isolates were detected by CarbaNP and 46 out of 47 were detected by MHT with one isolate negative for both, which could be due to mucoid phenotype/poor carbapenemase activity Similarly among bla_{NDM} producers, 19 out of 20 were detected by Carba NP and 18 among 19 were detected by MHT. Both the results are not in concurrence with the CLSI statement as only 11 % $bla_{oxa-48\ like}$producers can be detected by CarbaNP, while we detected 74% and only 11% of bla_{NDM} can be detected by MHT, as we detected 95% of isolates. *,†CarbaNP positivity may be due to bla_{NDM} co-production
Klebsiella spp. (n=14)	-	+	1	11	1; 1	0	Carba NP Fails in the detection of 11 isolates of $bla_{oxa-48like}$ producers, while MHT aids in the detection
Klebsiella spp. (n=2)	-	-	-	1	-	1‡	bla_{OXA-48} like negativity could be due to mucoid phenotype/poor carbapenemase activity ‡Could be due to other resistant mechanisms
Klebsiella spp. (n=1)	+	-	1	-	-	-	bla_{NDM} producer fails to be detected by MHT while CarbaNP detected
Total no tested: *E.coli (n=57)*							
E.coli (n=46)	+	+	28	8	5	5‡	In *E.coli*, bla_{NDM} were predominant. Among 33 bla_{NDM} producers, 31 were detected by CarbaNP; out of 10 $bla_{Oxa-48\ like}$ enzymes, 9 were picked up by CarbaNP. Same numbers were picked up by MHT as well
E.coli (n=5)	-	+	1	1	3	-	CarbaNP fails to detect 1 $bla_{OXA-48\ like}$ and 1 bla_{NDM} producer
E.coli (n=4)	+	-	3	1	-	-	bla_{NDM} producer fails to be detected by MHT while CarbaNP detected
E.coli (n=2)	-	-	1	-	-	1‡	bla_{NDM} negativity could be due to mucoid phenotype ‡Could be due to other resistant mechanisms

[Table/Fig-2]: Results of carbapenem resistance characterization by phenotypic and molecular methods for *E.coli* and *Klebsiella spp.*

	CarbaNP	bla_{NDM}	bla_{VIM}	$bla_{OXA-48\ like}$ bla_{NDM} +bla_{OXA-23} *	Class D bla_{OXA} (23†& 24‡)	No Carbapenemases genes detected	Remarks
P. aeruginosa: total tested (n=60)							
P. aeruginosa (n=23)	+	9	9	1	Not done	4§	Excellent sensitivity in detecting MβL producers §could be other MβL genes which were not included in the multiplex PCR panel/any other mechanisms
P. aeruginosa (n=37)	-	1	2	-	Not done	34‖	Fails to detect one bla_{NDM} and two bla_{VIM} isolates may be due to mucoid phenotypes ‖ majority of isolates were negative for CarbaNP and PCR, indicating the prevalence of intrinsic mediated resistance mechanisms such as *ampC*+porins or efflux pumps
A. baumannii: total tested (n=58)							
A. baumannii (n=47)	+	-	-	9*	38†	-	†CarbAcineto NP protocol is better for detection of class D bla_{OXA-23} producers
A. baumannii (n=11)	-	-	-	-	11‡	-	‡Out of 49 bla_{OXA-23} producers, CarbAcineto NP fails to pick up 11 isolates

[Table/Fig-3]: Results of carbapenem resistance characterization by phenotypic and molecular methods for *P. aeruginosa and A. baumannii.*

CarbAcineto NP was positive in 81% (*n=47/58*) of *A. baumannii.* MHT was found to be positive in 89% (*n=51*/57) of *E.coli* and 81% (*n*=69/85) of *Klebsiella* spp. [Table/Fig-2,3].

The multiplex PCR for the detection of carbapenemase genes revealed the presence of 57 $bla_{OXA-48like}$ producers, 53 bla_{NDM} producers, 11 bla_{VIM} producers and few co-producers of bla_{NDM}+$bla_{OXA-48like}$, bla_{NDM}+bla_{VIM} and bla_{NDM}+bla_{OXA-23} as listed in [Table/Fig-3,4]. Synergism testing of imipenem plus cloxacillin for Carba NP negative isolates of *P. aeruginosa* revealed that, out of 37 isolates, 24 were positive for *AmpC* plus porin loss detection.

The overall sensitivity and specificity of CarbaNP were found to be 84 % and 100% respectively. The sensitivity and specificity for MHT was found to be 96 % and 100 % for $bla_{OXA-48\ like}$ producers and 90% and 100% for bla_{NDM} producers. While, sensitivity and specificity of CarbaNP shows 77% and 100% for $bla_{OXA-48\ like}$ producers and 94% and 100% for bla_{NDM} producers. On the other hand, CarbAcineto NP showed 81% sensitivity and 100% specificity rates [Table/Fig-4].

DISCUSSION

In comparison with the CLSI recommendations and the study finding, there is a significant variation observed in the sensitivity and specificity. In case of MHT, CLSI states that there is >90%

Sensitivity and Specificity rates				
		CLSI	India (present study)	
	Carbapenemases	Sensitivity and specificity	Sensitivity	Specificity
Modified Hodge Test	$bla_{OXA-48like}$	>90%	96	100
	bla_{NDM}	Up to 11%	90	100
Carba NP	$bla_{OXA-48like}$	Up to 11%	77	100
	bla_{NDM}	>90%	94	100
CarbAcineto NP	Class D Carbapenemase: $bla_{Oxa23,24,58}$ which is more predominant in *Acinetobacter spp.*	Non committal	81	100

[Table/Fig-4]: Sensitivity and specificity of carbapenemase detection assays

sensitivity and specificity for bla_{KPC} carbapenemase and 11% for metallo β-lactamases. In this study, we however found a sensitivity of 96% and specificity of 100% for non metallo β-lactamases. In addition, we found 90% sensitivity for bla_{NDM} like producers in contrast to 11% reported by CLSI.

Likewise, for CarbaNP test, CLSI states the sensitivity and specificity of >90% for metallo β-lactamases and up to 11% for bla_{OXA-48} like producers. Although we found 94 % sensitivity for metallo β-lactamases and 77% sensitivity for bla_{OXA-48} like producers unlike 11% reported by CLSI.

Upon testing the carbaNP negative *P.aeruginosa* for porin loss plus AmpC hyper producers, using imipenem with cloxacillin showed 65% (n=24/37) were positive, while 35 % of (n=13/37) isolates were still negative, which may be due to efflux pumps or loss of porins. These results are in accordance with previously reported studies, in which carbapenem resistance due to carbapenemases is found to be less in non-carbapenemase producers beacuse of porin loss and ampC hyper-production [26]

Interestingly, CLSI does not mention CarbAcineto NP. This gives an even better sensitivity and specificity of 81% and 100% respectively than CarbaNP test. The information derived from this study varies extensively when compared to the CLSI observations, indicating the influence of the type of carbapenemase prevalent in a geographic region and the suitable methodology used for enhanced detection of carbapenemase.

Remarkably, EUCAST neither recommends MHT or CarbaNP, but rather recommends specific enzyme based detection methods for the detection of resistance mechanisms such as using aminophenyl boronic acid, cloxacillin and EDTA for different classes of carbapenemases [27]. It is worthwhile to evaluate and compare these methodologies to with CLSI recommendations for any advantage for Indian settings as mentioned in [Table/Fig-5].

Moreover, studies have evaluated the performance of MHT in detecting metallo β-lactamases and found the sensitivity and specificity rates ranging from 58 to 78% and 57% to 100% respectively [28,29]. This is much higher than that mentioned by CLSI. Furthermore, the addition of Zinc sulfate to the test medium increases the sensitivity of the bla_{NDM} detection from 50% to 85.7%, but lacks specificity [11]. In addition, Neo-Sensitabs has also been evaluated and shown to be more efficient in detecting carbapenemases [30].

In spite of CarbaNP being simple and easy, its downside includes false positive and false negatives results due to the change in the pH of the test solution, the inoculum size, time of incubation, test isolate growth medium where the inoculum was taken, mucoid phenotypes and presence of class $bla_{OXA-48\ like}$ enzymes with low carbapenemase activity [31,32]. In addition, the colour change of red to orange-yellow must be detected to interpret the test and thus it is dependent on the observer. These conditions should be taken into consideration when interpreting the results. Finally in terms of time, the CarbaNP takes two hours, while the MHT requires the 24 hours time period for the carbapenemase detection.

This study reports 90% sensitivity and specificity for $bla_{oxa-48\ like}$ enzymes by MHT and >90% sensitivity and specificity for bla_{NDM} producers by CarbaNP method. Even though MHT was estimated by CLSI to give a sensitivity of 11% for bla_{NDM} producers, much higher rates were found in Indian settings as in this study where it was around 90% sensitive and 100% specific. Similarly, $bla_{oxa-48\ like}$ enzymes detection by CarbaNP was >70% sensitivity and specificity while CLSI observation was only 11%. Although, CLSI recommends CarbaNP for *A.baumannii,* it is well known that CarbAcinetoNP has a higher sensitivity and specificity for *A.baumannii* [14].

LIMITATIONS

Other carbapenemase encoding genes were not looked for the isolates that are resistance to carbapenem but negative for the carbapenemase genes tested in PCR.

	MIC				Recommendations for carbapenemase detection				
	CLSI (2015)		EUCAST (2015)		CLSI (2015)		EUCAST		
	≤S	≥R	≤S	≥R					
Enterobacteriaceae					Modified Hodge Test (only for *Enterobacteriaceae*)	Carba NP Test	Synergism testing of meropenem (M) with APBA, cloxacillin and EDTA		Carba NP Test
Doripenem	1	4	1	2	>90% sensitivity for bla_{KPC} producers	>90% sensitivity for bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{SPM}, bla_{SME}	M+APBA	Class A carbapenemase	Recommended only for *Enterobacteriaceae*
Imipenem	1	4	2	8			M+APBA and M+Cloxacillin	AmpC+ Porin loss	
Meropenem	1	4	2	8	11 % sensitivity for bla_{NDM} producers	11% sensitivity for $bla_{OXA-48\ like}$ producers	M+EDTA	MBLs	Less sensitive for bla_{OXA-48} like producers
Ertapenem	0.5	2	0.5	1			No synergism with any of the combinations	$bla_{OXA-48\ like}$ and ESBL+Porin loss	
P. aeruginosa									
Doripenem	2	8	1	2	Not recommended	Same as of *Enterobacteriaceae*	Not Recommended		
Imipenem	2	8	4	8					
Meropenem	2	8	2	8					
A.baumannii									
Doripenem	2	8	1	2	Not recommended	Although, EUCAST does not recommend CarbaNP, Nordmann and Poirel et al., recommended the use of CarbAcineto NP for the detection of carbapenemases in *A. baumannii*			
Imipenem	2	8	2	8					
Meropenem	2	8	2	8					

[Table/Fig-5]: Interpretative criteria for carbapenems according to CLSI and EUCAST guidelines.

CONCLUSION

Detection of carbapenem resistant organism (CRO) using different methodologies demonstrates variations in the sensitivity and specificity across different geographical regions. Overall, it was evident that MHT is comparatively better for the detection of class D Oxa enzymes but not for metallo β lactamases. On the contrary, CarbaNP is excellent rapid test in detecting metallo β lactamases but not for class D Oxa enzymes. Henceforth, it is advisable to screen the isolates through CarbaNP for the rapid detection, followed by MHT for any region where class D Oxa-enzymes are more prevalent. Eventually, combined testing of both Carba NP and MHT will certainly help in detecting the most of the carbapenemases. Moreover, this study signifies the importance of knowing the predominant carbapenemase enzymes seen in a specified geographical region before deciding upon a suitable phenotypic screening technique. Further work should investigate the EUCAST proposed carbapenemase detection algorithm to establish whether this better suits the Indian setting.

ACKNOWLEDGEMENT

The authors thank Indian Council of Medical Research (ICMR) and Fluid Research Grant (CMC) for provided funding to carry out this evaluation as a part of Antimicrobial Surveillance Study. We also thank Dr. Laura Nabarro (Public Health England) for manuscript proof reading and suggestions.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Aug 24, 2015**
Date of Peer Review: **Oct 31, 2015**
Date of Acceptance: **Apr 12, 2016**
Date of Publishing: **Jul 01, 2016**