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EVALUATION OF THE PERFORMANCE OF SIMPLE, RELIABLE AND USER-FRIENDLY PHENOTYPIC METHODS FOR THE DETECTION OF CARBAPENEMASE, AMPC B-LACTAMASE AND ESBL PRODUCTION IN CARBAPENEM RESISTANT GRAM-NEGATIVE BACILLI

Microbiology

Dr. Soma Sarkar — Associate Professor, Department of Microbiology NRS Medical College, Kolkata, 138 AJC Bose Road, Kolkata 700014, India

Dr. Shanta Dutta — Director, National Institute of Cholera and Enteric Diseases P-33, C. I. T. Road, Scheme - XM, Beliaghata, Kolkata 700010, India

Dr. Anjum Namhata — Senior Resident, Department of Microbiology NRS Medical College, Kolkata 138 AJC Bose Road, Kolkata 700014, India

Dr Manideepa SenGupta* — Prof. and Head, Department of Microbiology, Medical college and Hospital, Kolkata 88 College street, Kolkata-700073, India *Corresponding Author

ABSTRACT

Dissemination of carbapenem resistant GNB (CRGNB) is a major public health concern.Rapid identification of carbapenemase producing GNB is necessary to initiate proper antibiotic regimen and also to prevent dissemination of antibiotic resistance. Although genotypic detection tests remain the gold standard for carbapenemase, their costs in a resource poor laboratory limits its use. In this study, the rapidity, sensitivity and specificity, positive predictive value and negative predictive value of phenotypic methods were compared to genotypic methods to detect carbapenemase in 539 CRGNB. The sensitivity of Combined disk synergy test (CDST) by using EDTA, Double Disc Synergy Test (DDST) + EDTA, MBL E – test, mCIM (Modified Carbapenemase Inactivation Method), Rapidec Carba was 70.28%, 74.06%, 76.07%, 86.15%, 89.17% respectively and specificity was 90.85%, 81.69%, 90.14%, 97.89%, 100% respectively. AmpC hyper producer were 24 (16.90%) among 142 non-carbapenemase producing CRGNB. The sensitivity of ESBL production w 69.57% with ceftazidime plus clavulanic acid (CAZ-CA) and coproduction of ESBL and carbapenemase was 53.06%.

KEYWORDS

CRGNB, Carbapenemase, Phenotypic methods

INTRODUCTION

The emergence and rapid expansion of carbapenem resistant gram-negative bacteria (CR-GNB) is a global public health threat. Carbapenemases degrade broad-spectrum β-lactams including carbapenems, located on mobile genetic elements, facilitate the spread of resistance genes between organisms. [1] Carbapenemase enzymes include class A carbapenemases (KPC types), class B or metallo-beta-lactamases (MBLs e.g. VIM, IPM, and NDM types), and class D oxacillinases (e.g., OXA-48-like enzymes). [2] In addition, decreased susceptibility to carbapenems in Gram negative bacilli may be also be caused by either extended spectrum beta-lactamases (ESBLs) or AmpC enzymes combined with drug-impermeablity, due to loss of porins.[3] Carbapenem-resistant GNB, reported mostly in hospital-associated infections, is associated with more than 50% mortalities which poses a great challenge in patient care units. [4] Rapid identification of carbapenemase producing GNB is necessary to initiate proper antibiotic regimen and also to prevent dissemination of antibiotic resistance. Genotypic methods for the detection of carbapenemase genes are costly, may require significant expertise and most of the laboratories cannot afford this. [5] Thus, rapid, cost-effective, simple and reliable phenotypic procedures are required which can be comparable closely with the results of genotypic methods. So, our study aimed to evaluate various phenotypic methods for detection of carbapenemase production in genetically proven carbapenemase producing strains prevalent in Eastern India.

MATERIALS AND METHODS

Various types of samples like blood, urine, pus, sputum and endo-tracheal (ET) aspirates collected from different wards were cultured and anti-microbial susceptibility testing of culture positive samples were done by Vitek2 system. CRGNB were identified and screening for carbapenemase was done according to MIC breakpoints for ertapenem (2 µg/ml) in cases of *Enterobacteriaceae* (as by CLSI guideline [6]) and MIC breakpoints of meropenem (8µg/ml) was used in *Acinetobacter* and *Pseudomonas spp*. Screened CRGNB were genetically analysed and various phenotypic tests (described below) were also performed with that strains

Genotypic methods for detection of carbapenemase production

The presence of carbapenemase encoding genes was determined by multiplex PCR, [7] using primers (ReadyMade™ *Primers,* Integrated DNA Technologies) targeting *bla* VIM, *bla* IMP, *bla* KPC, *bla* OXA-48, and *bla* NDM which are prevalent in India. For amplification, 4μL of template DNA was added to a 16μL of 2 × master mix multiplex PCR Kit (Qiagen Benelux, Antwerp, Belgium). PCR was performed under the following conditions: 15 min at 95^{0}C and 30 cycles of 30 s denaturation at 94^{0}C; 90 s annealing at 57^{0}C and 90 s elongation at 72^{0}C; and a final elongation step at 72^{0}C for 10 min. Quality control was performed with each run using positive control strains of *Klebsiella pneumoniae* ATCC BAA2156 for NDM-1, *Klebsiella pneumoniae* NCTC13442 for OXA-48, *Klebsiella pneumoniae* ATCC BAA1705 for KPC.privacy policy

Phenotypic methods for detection of carbapenemase production

1.Combined disk synergy test (CDST) by using two different β-lactamase inhibitors phenylboronic acid (PBA) and EDTA

The stock solution of PBA [8] in the concentration of 20 mg/ml was prepared by dissolving 120 mg of PBA (HIMEDIA, Mumbai, India) in 3 ml of dimethyl sulfoxide (DMSO) followed by adding 3 ml of sterile distilled water. A 0.5 M EDTA solution [9] was prepared by dissolving 186.1 g of disodium EDTA.2H_2O in 1,000 ml of distilled water. The pH was adjusted to 8.0. Three ertapenem (ETP), imipenem (IPM), and meropenem (MRP) discs were placed in rows on the Mueller Hinton agar (MHA) plate seeded with 0.5 McFarland inoculum of the test strain. Then, 10 µL of EDTA (750 µg) and 20 µL (400 µg) of PBA were added to the first and third discs, respectively. A difference of ≥5 mm in zone diameter (around the discs) between the discs containing the PBA and EDTA solutions and that containing carbapenems alone was considered positive for KPC and MBL production respectively [Fig 1]

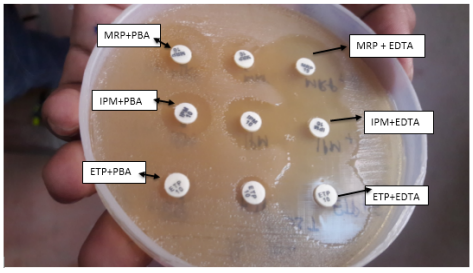

Fig.1: CDST showing a difference of ≥5 mm in zone diameter between the discs containing carbapenem + PBA/ EDTA solutions and that containing carbapenems alone was considered positive for KPC and MBL production respectively

2. *Double Disc Synergy Test (DDST)* [10][11]

EDTA and PBA discs were prepared by incorporating 10 µl of 0.5 M solution of EDTA solution and 20 µl PBA on blank discs (6 mm in diameter, Whatman filter paper no.1) and was placed 20 mm apart from the carbapenem disks on MHA plates seeded with test strains. After overnight incubation at 37° C, a zone of synergy between the carbapenem disc and EDTA/PBA was taken as a positive result [Fig 2]

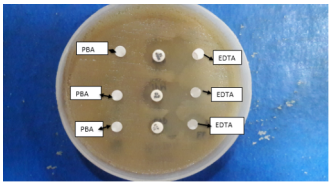

Fig.2: DDST showing a zone of synergy between the carbapenem disc and EDTA/PBA on blank disc was taken as a positive result

3. *MBL E – test* [12]

MBL E test strips (HIMEDIA, INDIA) is a double-sided strip consisting of meropenem (MRP) (4 to 256 µg/ml) on one side and meropenem plus EDTA (1to 64 µg/ml) on the other. The test organisms were adjusted to a 0.5 McFarland turbidity standard and inoculated on MHA plates. The E-test was done according to manufacturer's instructions. The presence of MBL is indicated by a reduction of MRP MIC ≥3 twofold dilutions in the presence of EDTA or the appearance of phantom zones, or deformation of ellipse [Fig 3]

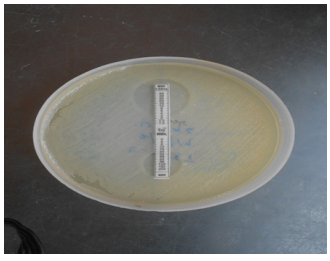

Fig.3: MBL-E Test showing reduction of MRP MIC ≥3 twofold dilutions in the presence of EDTA as compared to MRP alone indicating carbapenemase production

4. *Modified Hodge Test (MHT)* [13]

A culture suspension of *Escherichia coli* ATCC 25922 adjusted to 0.5 Mac Farland standard was inoculated on the surface of MHA plate. After drying, 10 µg meropenem disc was placed at the centre of the plate and the test strains with one positive control (*Klebsiella pneumoniae* ATCC 1705) and one negative control (*K. pneumoniae* ATCC 1706) were streaked from the edge of the disc to the periphery of the plate. After overnight incubation at 37° C, the presence of a cloverleaf shaped zone of inhibition by the test strain was considered as positive [Fig 4]

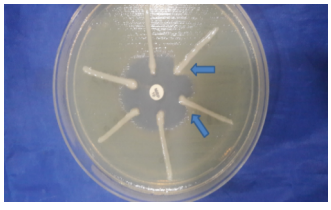

Fig.4: Clover leaf shaped zone in **Modified Hodge test** indicating carbapenemase production by the test strain (marked by an arrow)

5. *mCIM (Modified Carbapenemase Inactivation Method)* [6]

1-µl loopful of *Enterobacteriaceae* isolates and 10-µl loopful of *Pseudomonas* and *Acinetobacter spp* were emulsified with 2 ml of TSB (Trypticase soy broth). A meropenem disc (10 µg) was added in the TSB-organism solution and then incubated for 4 h +15 min at 37°C. A lawn culture of *Escherichia coli* ATCC 25922 was made by 0.5 Mc-Farland suspension on a MHA plate and the meropenem disc was removed from the TSB-organism broth and placed over the lawn. After overnight incubation at 37°C, the result was interpreted based on the zone diameter of the meropenem disc and considered positive if it is 6 to 15 mm or presence of pinpoint colonies within a 16-18mm zone, indeterminate if 16 to 18 mm or ≥19 mm with presence of pinpoint colonies within the zone and negative if ≥19 mm (clear zone). eCIM interpretation was done only when mCIM was positive. A ≥ 5mm zone diameter in eCIM than that of mCIM was considered MBL positive [Fig 5]

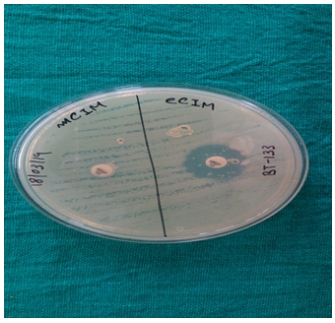

Fig.5: mCIM showing the zone diameter of meropenem disc was considered positive. A ≥ 5mm zone diameter in eCIM than that of mCIM was considered MBL

6. *Rapidec Carba NP*

This ready to use test was performed according to the brochure provided with the kit. The Carba NP [14] test is based upon that if the bacterial carbapenemase enzyme hydrolyzes imipenem in the presence of zinc, it causes a change in pH which causes phenol red to change its colour from red to yellow. The time required for the test was between 30 minutes to 2 hours and result were obtained on the same day [Fig.6]

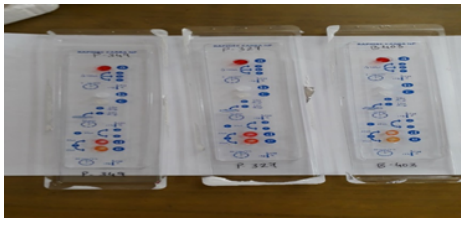

Fig.6: Rapidec Carba NP: As the colour changes from red to yellow, light orange, orange or dark orange in well e, the result is positive in right and left side but colour remains red in the middle, so it is negative

Phenotypic detection of AmpC production among CRGNB

Cefoxitin-Cloxacillin Double Disc Synergy test (CC-DDS) [15]

Disks containing 30 µg of cefoxitin (CX) and 30 µg of cefoxitin plus 200 µg of cloxacillin disk (CXX) were used (Hi Media, Mumbai, India). The test strains were inoculated on MHA using McFarland 0.5, followed by putting the discs with centres at least 24 mm apart and incubated at 37°C for 18-24 h. A difference in the cefoxitin-cloxacillin inhibition zones minus the cefoxitin alone zones of ≥4 mm was considered indicative for AmpC production [Fig.7]

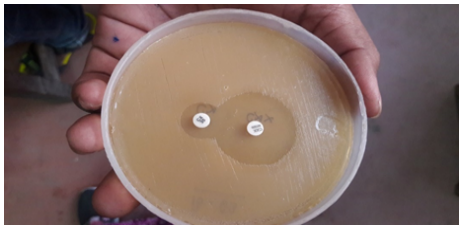

Fig.7: CC-DDS showing a difference in cefoxitin-cloxacillin inhibition zone minus the cefoxitin zone alone of ≥4 mm was considered indicative of AmpC production

Phenotypic detection of ESBL among CRGNB using the modified CLSI ESBL confirmatory test [16]

The test strains (0.5 McFarland) were lawned on MHA. Cefotaxime (CTX) and ceftazidime (CAZ) disks with or without clavulanic acid (CA) were placed on MHA plate, on which both PBA solution (20 µl) and EDTA solution (4µl of 0.5 M EDTA containing 292 µg of EDTA) were dispensed. After incubation at 37° C for 18 h, an augmentation of ≥5 mm in the growth-inhibitory zone diameter of either CTX-CA or CAZ-CA in combination with PBA and EDTA (CTX-CA-PBA-EDTA and CAZ-CA-PBA-EDTA, respectively) as compared to the zone diameter of CTX or CAZ disks containing PBA and EDTA (CTX-PBA-EDTA and CAZ- PBA-EDTA, respectively) was considered a positive result for ESBL production [Fig 8]

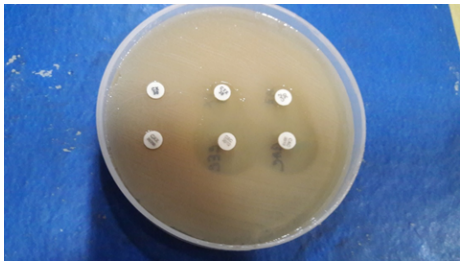

Fig.8: Phenotypic detection of ESBL among CRGNB showing an augmentation of ≥5 mm in the growth-inhibitory zone diameter of either CTX-CA or CAZ-CA compared to CTX or CAZ alone is considered as positive

Statistical analysis:
All calculations were done with SPSS statistics software version 20 (IBM Corporation, Armonk, NY).

RESULTS

Out of 539 nonduplicate carbapenem resistant clinical isolates of GNB, 242 *K. pneumoniae*, 23 *Enterobacter cloacae*, 161 *Escherichia coli*, 29 *Citrobacter spp*., 11 *Proteus spp*., 50 *Acinetobacter spp*. and 23 *Pseudomonas aeruginosa* were included in the study and screened for carbapenemase production.

Genotypic methods for detection of carbapenemase production
The presence of 397 carbapenemase encoding genes of various classes with their coproduction (NDM, OXA 48, OXA 23, NDM +OXA 48, NDM +OXA 23) was detected by multiplex PCR [Table 1]. The results of different phenotypic tests of these 397 genotypically characterised isolates were shown on Table 1. Phenotypic tests were also performed in 142 non-carbapenemase producing CRGNB.

Combined disk synergy test (CDST) by using two different β-lactamase inhibitors phenylboronic acid (PBA) and EDTA
Out of 397 carbapenemase producing CRGNB (CP-CRGNB), CDST using EDTA detected 279 (70.28%) CP-CRGNB correctly and 13 was falsely positive in respect to PCR. Out of 142 non-CP-CRGNB, 129 (90.84%) non-CP-CRGNB was detected reliably by this method but 118 was false negative. Though CDST using PBA detected 120 (30.22%) correctly as CP-CRGNB and 105 (73.94 %) as non-CP-CRGNB, it detected 2 KPC (100%) reliably.

Double Disc Synergy Test (DDST)
Out of 397 carbapenemase producing CRGNB (CP-CRGNB), DDST using EDTA detected 294 (74.05%) CP-CRGNB correctly and 26 was falsely positive in respect to PCR. 116 (81.69%) non-CP-CRGNB was detected correctly by this method and 103 was false negative. DDST using PBA detected KPC effectively (100%).

MBL E – test
MBL E-test detected 302 (76.07%) CP-CRGNB and 128 (90.14%) non-CP-CRGNB correctly among 397 carbapenemase producing CRGNB (CP-CRGNB) and 142 non-carbapenemase producing CRGNB (non-CP-CRGNB).

Modified Hodge Test (MHT)
Modified Hodge Test (MHT) detected 216 (54.40%) CP-CRGNB and 110 (77.46%) non-CP-CRGNB correctly among 397 carbapenemase producing CRGNB (CP-CRGNB) and 142 non-carbapenemase producing CRGNB (non-CP-CRGNB).

mCIM (Modified Carbapenemase Inactivation Method)
The mCIM had an overall sensitivity and specificity of 86% and 98% respectively, for detection of CP-CRGNB but was only able to identify 80% of OXA-48-types and 90% of NDM producers.

Rapidec Carba NP
Rapidec Carba NP test was able to detect 354 (89.16%) CP-CRGNB and 142 (100%) non-CP CRGNB correctly. 70% of OXA-48-types and 93.36% of NDM producers among 397 CP-CRGNB was detected by this method.

Table 1: Number of carbapenemase producing isolates by MHT, CDST, DDST, MBL E Test, mCIM and Rapidec Carba NP test in comparison with 397 genotypically characterised carbapenemase-positive isolates

Carbapenemase production by Genotypic methods (n=397)		MHT	CDST		DDST		MBL-E TEST	mCIM	RAPIDEC CARBA NP TEST
			USING CARBAPENEM DISC WITH CARBAPENEM+EDTA	USING CARBAPENEM DISC WITH CARBAPENEM+PBA	USING CARBAPENEM DISC WITH EDTA	DDST USING CARBAPENEM DISC WITH PBA			
KPC	2	2	0	2	0	2	0	2	2
NDM	226	116 (51.32%)	169 (74.77%)	98 (43.36%)	179 (79.20%)	100 (44.24%)	191 (84.51%)	203 (89.82%)	211 (93.36%)
OXA-48	30	20 (66.67%)	19 (63.34%)	7 (23.34%)	20 (66.67%)	9 (30%)	12 (40%)	24 (80%)	21 (70%)
OXA- 23	32	16 (50%)	14 (43.75%)	0	14 (43.75%)	0	21 (65.62%)	26 (81.25%)	23 (71.87%)
NDM +OXA 48	90	54 (60%)	66 (73.34%)	11 (12.23%)	69 (76.67%)	15 (16.67%)	64 (71.12%)	73 (81.12)	83 (92.23%)
NDM +OXA 23	17	8 (47.05%)	11 (64.70%)	2 (11.76%)	12 (70.58%)	2 (11.76%)	14 (82.35%)	14 (82.35%)	14 (82.35%)
397		216 (54.41%)	279 (70.28%)	120 (30.22%)	294 (74.06%)	128 (32.24%)	302 (76.07%)	342 (86.15%)	354 (89.17%)

Abbreviations: KPC-Klebsiella pneumoniae carbapenemases, OXA-Oxacillinases; NDM-New Delhi metallo-β-lactamases

Phenotypic detection of AmpC hyper-production and ESBL among CRGNB
AmpC hyperproducer were 24 which was 4.45% among 539 CRGNB and 16.90% among 142 non-CP-CRGNB. The sensitivity of ESBL production was more (69.57%) with ceftazidime plus clavulanic acid (CAZ-CA) than cefotaxime plus clavulanic acid (CTX-CA) (59.55%) [Table 2].

Table 2: Results of phenotypic AmpC test, ESBL test in association with carbapenemase production in 539 CR isolates from different clinical samples

Sample	CR isolates	Carbapenemase producing GNB by Genotypic methods	PHENOTYPIC AMPC POSITIVE	PHENOTYPIC ESBL POSITIVE CAZ-CAC	CTX-CEC	ESBL and Carbapenemase co-producer
BLOOD Neonatal septicaemia cases	96	62 (64.58%)	4 (4.16%)	58 (60.41%)	51 (53.12%)	48 (50%)
Adult septicaemia	48	38 (79.16%)	3 (6.25%)	37 (77.08%)	32 (66.67%)	26 (54.17%)
URINE	193	164(84.97%)	7 (3.62%)	151(78.23%)	130 (67.35%)	123 (63.73%)
PUS	128	90 (70.31%)	6 (4.68%)	82 (64.06%)	70 (54.68%)	68 (53.12%)
SPUTUM	40	20 (50%)	2 (5%)	26 (65%)	20 (50%)	11 (27.5%)
ET	34	23 (67.64%)	2 (5.88%)	21 (61.76%)	18 (52.94%)	10 (29.41%)
TOTAL	539	397 (73.65%)	24 (4.45%)	375 (69.57%)	321 (59.55%)	286 (53.06%)

DISCUSSION

Carbapenems were used as a last resort, until recently CRGNB have emerged posing a great threat to mankind. In our study total 539 carbapenem resistant isolates were analyzed among which 397 (73.65%) were genetically proven carbapenemase producer. It is similar with studies by John Osei Sekyere et al (2016) [17] and Asifa et al

(2018) [18] which showed 85.41% and 74.54% carbapenemase producing isolates respectively among carbapenem resistant strains. Maryam Al Tamami et al (2016) [4] showed 48.3% carbapenemase producing isolates which are much lower than our study. A comparative evaluation was done among the phenotypic methods in Table 3.

Table 3: Comparative evaluation of the phenotypic tests

	MHT	CDST (Using EDTA)	DDST (Using EDTA)	MBL-E TEST	mCIM	Rapidec Carba NP
Sensitivity (95% CI)	**54.41%** (49.37% to 59.38%)	**70.28%** (65.52% to 74.73%)	**74.06%** (69.45% to 78.30%)	**76.07%** (71.56% to 80.19%)	**86.15%** (82.35% to 89.39%)	**89.17%** (85.69% to 92.05%)
Specificity (95% CI)	**64.08 %** (55.61% to 71.96%)	**90.85 %** (84.85% to 95.03%)	**81.69% (**74.33% to 87.68%)	**90.14% (**84.01% to 94.50%)	**97.89 %** (93.95% to 99.56%)	**100.00%** (97.44% to 100.00%)
Positive Predictive Value	**80.90%** (76.96% to 84.30%)	**95.55%** (92.72% to 97.31%)	**91.88%** (88.83% to 94.15%)	**95.57%** (92.90% to 97.27%)	**99.13%** (97.38% to 99.71%)	**100.00%**
Negative Predictive Value	**33.46 %** (29.92% to 37.19%)	**52.23%** (48.23% to 56.20%	**52.97%** (48.38% to 57.50%)	**57.40 %** (52.86% to 61.82%)	**71.65 %** (66.39% to 76.38%)	**76.76%** (71.35% to 81.41%)
Accuracy	**56.96%** (52.66% to 61.18%)	**75.70% (**71.85% to 79.26%)	**76.07%** (72.23% to 79.61%)	**79.78% (**76.13% to 83.09%)	**89.24%** (86.31% to 91.73%)	**92.02%** (89.40% to 94.17%)
Limitations and strength	False positive results may occur in ESBL or AmpC hyperproducer and false negative may occur in cases of NDM producers	Inhibitor based assay. Simple to perform. Detects MBL reliably than OXA.	Simple to perform. Detects MBL reliably than OXA	Estrips are costly.	More reliably applicable to Enterobac- teriaceae Requires overnight incubation.	Special regents are needed. Point of care test, Cost /test Rs 300. OXA types are not consistently detected
Turnaround time	Results will be ready on next day	Results will be ready on next day	Results will be ready on next day	Results will be ready on next day	Results will be ready on next day	Results will be ready in (≤2hrs).

All the phenotypic tests were less sensitive for the detection of OXA-48-type producers. Due to increase in false positive and false negative results in MHT, it is no longer recommended by CLSI [6] or EUCAST. 142 (26.34%) isolates which were proven negative genotypically, were also tested for all the phenotypic methods including Amp C and ESBL detection methods to observe the specificity of the phenotypic tests as well as production of ESBL or Amp C hyper producers.

CONCLUSION

Rapid and reliable detection of carbapenemase producers is critical to implement timely contact isolation as CP-CRGNB can widely disseminate in health care settings if proper infection control practices are not followed. Although genotypic detection remains the gold standard for carbapenemase, their costs associated with these tests in a resource poor laboratory limits its use. Considering the performances of all the phenotypic methods for detecting carbapenemase-producing GNB, Rapidec Carba NP and mCIM were the most satisfactory simple, easy, rapid and reliable method.

REFERENCES

1. McMullen A, Yarbrough M, Wallace M, Shupe A, Burnham C. Evaluation of Genotypic and Phenotypic Methods to Detect Carbapenemase Production in Gram-Negative Bacilli. Clinical Chemistry. 2017; 63(3):723-730.
2. Pandurangan S, BegumEsak S, Narayanasamy A. Phenotypic Detection Methods of Carbapenemase Production in Enterobacteriaceae. Int.J.Curr.Microbiol.App.Sci. 2015; 4(6): 547-552
3. Bartolini A, Frasson I, Cavallaro A, Richter S, Palù G. Comparison of phenotypic methods for the detection of carbapenem non-susceptible Enterobacteriaceae. Gut Pathogens. 2014; 6(1):13.
4. AlTamimi M, AlSalamah A, AlKhulaifi M, AlAjlan H. Comparison of phenotypic and PCR methods for detection of carbapenemases production by Enterobacteriaceae. Saudi J Biol Sci. 2017; 24(1):155–161
5. Tamma P, Opene B, Gluck A, Chambers K, Carroll K, Simner P. Comparison of 11 Phenotypic Assays for Accurate Detection of Carbapenemase-Producing Enterobacteriaceae. Journal of Clinical Microbiology. 2017; 55(4):1046-1055.
6. CLSI M100-S29.Performance Standards for Antimicrobial Susceptibility Testing. Twenty-nine Informational Supplement. CLSI Wayne, USA; Jan 2019
7. Bogaerts P, Rezende de Castro R, Mendonca R de, Huang T-D, Denis O, Glupczynski Y. Validation of carbapenemase and extended-spectrum b-lactamase multiplex endpoint PCR assays according to ISO 15189. J Antimicrob Chemother. 2013; 68: 1576–1582
8. Girgis S A, Othman H B, Kassem N N, Abdou S M. Evaluation of Boronic Acid Disk for Detection of Klebsiella pneumoniae Carbapenemase in Klebsiella pneumoniae. Int.J.Curr.Microbiol.App.Sci (2015) 4(5): 772-783
9. Asthana S, Mathur P, Tak V. Detection of Carbapenemase Production in Gram-negative Bacteria. J Lab Physicians. 2014; 6(2):69–75.
10. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge Test and the Imipenem-EDTA Double-Disk Synergy Test for Differentiating Metallo-β-Lactamase Producing Isolates of Pseudomonas spp And Acinetobacter spp. J Clin Microbiol 2003;41: 4623-4626.
11. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwar H, Goto M. Convenient test for screening metallo-beta-lactamase producing gram negative bacteria by using thiol compounds. J Clin Microbiol 2000; 38:40-43.1
12. Khosravi Y, Loke M, Chua E, Tay S, Vadivelu J. Phenotypic Detection of Metallo-β-Lactamase in Imipenem-Resistant Pseudomonas aeruginosa. The Scientific World Journal. 2012; 2012:1-7.
13. Amjad A, Mirza Ia, Abbasi S, Farwa U, Malik N, Zia F. Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iran J Microbiol. 2011; 3(4):189–193.
14. Laurent Poirel, Patrice Nordmann. Rapidec Carba NP Test for Rapid Detection of Carbapenemase Producers. Journal of Clinical Microbiology Aug 2015, 53 (9) 3003-3008
15. Fatin Izzati Mohd Khari, Rina Karunakaran, Roshalina Rosli, Sun Tee Tay. Genotypic and Phenotypic Detection of AmpC β-lactamases in Enterobacter spp. Isolated from a Teaching Hospital in Malaysia.PLoS One. 2016; 11(3): e0150643.
16. Poulou A, Grivakou E, Vrioni G, et al. Modified CLSI extended-spectrum β-lactamase (ESBL) confirmatory test for phenotypic detection of ESBLs among Enterobacteriaceae producing various β-lactamases. J Clin Microbiol. 2014; 52(5):1483–1489.
17. Sekyere J, Govinden U, Essack S. Comparison of Existing Phenotypic and Genotypic Tests for the Detection of NDM and GES Carbapenemase- Producing Enterobacteriaceae. Journal of Pure and Applied Microbiology. 2016; 10(4):2585-2591.
18. Bhat A, Kakru D, Farooq S, Angmo D, Suhail M, Nazir S et al. COMPARISON OF PHENOTYPIC AND GENOTYPIC METHODS FOR DETECTION OF CARBAPENEM RESISTANCE IN AN INDIAN TERTIARY CARE HOSPITAL. International Journal of Advanced Research. 2018; 6(5):1452-1457.