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Original Research Article

Assessment of Rapidec Carba NP Test, as Screening Test to Detect Carbapenemase, Specially Metallo B Lactamase Producing Gram Negative Bacteria at Tertiary Care Centre

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ABSTRACT

Rapidec Carba NP test is recommended by Clinical laboratory Standard institute (CLSI) for detection of carbapenemase.
Metallo β lactamase (MBL), especially NDM1 are increasingly detected in Indian health care settings. Routine phenotypic test to detect MBL requires about 48 hours & other tests including molecular test are laborious & not rapid. So, present study was undertaken to assess the utility of Rapidec Carba NP test in detection of Carbapenemase & MBL producing Gram Negative bacilli in paediatric patients in comparison to phenotypic test.
Aim & Objective: To assess the utility of Rapidec Carba NP test to detect carbapenemase, specially, MBL producing Gram Negative Bacilli & carbapenemase to know the occurrence of MBL in paediatric patients.
Settings and Design: Prospective, laboratory based cross sectional study was conducted at Microbiology Laboratory of Tertiary Care centre.
Methods and Material: Fifty Carbapenem Resistance Gram negative isolates from paediatric patients identified by Vitek 2 Compact automation system were further tested for MBL production, using phenotypic test. All isolates including MBL positive were subjected to Rapidec Carba NP Test.
Statistical analysis used: Statistical package of social science (SPSS) version 22.0 software.
Results: 82% carbapenem positive isolates were detected by Rapidec Carba NP Test out of fifty isolates. Twenty isolates were identified as MBL positive, eighteen of which were correctly identified by Rapidec Carba NP test giving sensitivity of 90%.
Conclusions: With 90% sensitivity &Turnaround time of 30 minutes to 2 hours, Rapidec Carba NP test proves effective screening test in adjunct with other phenotypic test. Forty percent carbapenem resistant isolates were MBL positive in Paediatric patients.

Key Words: Rapidec Carba NP test, CLSI, Metallo-beta-lactamase, Paediatric.

INTRODUCTION

Carbapenemase are specific beta lactamase present in Gram Negative bacteria, with the ability to hydrolyse carbapenem, a broad spectrum antimicrobial agent used as last resort antibiotics to treat multidrug resistant infections & their production is most important cause of carbapenem resistance thus limiting the treatment options available. [1,2] Metallo-β-lactamase (MBL) are zinc dependant β-lactamases which can hydrolyse all β

lactamase except aztreonam. NDM1(New Delhi Metallo β lactamase) is recently discovered in 2008, from a patient treated in India & returning to Sweden.NDM1 positive isolates may carry additional resistance mechanism to amino glycosides, fluoroquinolones group of antibiotics thus limiting the treatment options in these infections leading to increase mortality. [3-8]

Routine testing may not always detect this mechanism. The production of MBL which are transmissible in another species results in wider spread of antibiotic resistance. [9-12]

Phenotypic Screening of MBL is useful to limit the spread of MBL infections in health care setting, if detected early as infection control measures like barrier precautions can be initiated without delay and to choose the apt antibiotic regimen resulting in decrease morbidity and mortality associated with these MDR infections. [13-16]

Indian prevalence of Metallo β-lactamase range from 70-90% [17] No data about performance characteristic of Rapidec Carba NP Test is available at our center for screening of carbapenemase positive isolates & as adjunct test for screening of MBL positive isolate. Clinical & Laboratory standards Institute (CLSI), 2015 incorporated Carba NP assay as screening test. (CLSI M100-S25). Rapidec Carba Np assay is novel inhibitor based biochemical assay developed in two versions by Nordmann & co-workers for detection of carbapenemase. Rapidec Carba Np test is based on Carba Np I, which do not discriminate among classes of carbapenemases so it's imperative to study its suitability in detection of class specific resistance in bacteria. [18] Present study was undertaken with purpose to assess suitability of Rapidec Carba NP test in detection of MBL class carbapenemase rapidly so infection control can be effectively implemented & antibiotic treatment can be designed accordingly with the aim to assess utility of Rapidec Carba Np test to detect carbapenemase, specially, MBL producing Gram Negative Bacilli in adjunct to phenotypic test & with objective to know the occurrence of MBL in paediatric patients.

MATERIALS AND METHODS

Prospective, observational, laboratory based study was conducted after obtaining Institutional Ethics Committee clearance. The study was conducted at Microbiology Laboratory of tertiary care centre. Fifty Gram Negative bacilli, isolated from paediatric population, identified as Carbapenemase resistant by Vitek 2 Compact were included in study. MBL production was further confirmed using EZY MIC^{Tm} (EM078) E. strips by HIGH-MEDIA with Imipenem alone & Imipenem with & without EDTA combination & HIGH-MEDIA Imipenem Disc with & without EDTA were used for MBL detection.

Phenotypic EZYMIC^{Tm} strips for MBL Detection: [19] Figure 1

Phenotypic MBL detection strip which is coated with mixture of Imipenem + EDTA (1-64 mcg/ml) & Imipenem (4-256mcg/ml) on single strip in a concentration gradient manner were used. The upper half of the strip has Imipenem + EDTA with highest concentration tapering downwards whereas half is similarly coated with Imipenem in a concentration gradient in a reverse direction.

Combined Disk test(Imipenem10µg+EDTA750 µg HIGH-MEDIA): Figure 2

Combined Disk test is done using Imipenem (10µg) & Imipenem+ ethylene diamine tetra acetic acid disc (Imipenem10µg+EDTA750 µg HIGH-MEDIA). [20-25]

When the Zone difference of combined disk i.e. Imipenem+EDTA & Imipenem alone was >7mm, it was considered as production of MBL. Procedure for Rapidec Carba NP test. [26]

The test is based on the principle described by Nordmann, Poirel, Dordet. It detects carbapenem hydrolysis by carbapenemase producing bacteria, resulting in Acid production causing change of colour of Ph Indicator phenol red. Test was done as per manufacturer instruction.10µl of inoculum equivalent to loop full of colonies taken from culture plate is mixed with cell, visual reading of Test strip was done initially after 30 minutes of incubation at 37ºC& later after 2 hours if required, of Control well without Imipenem to Reaction well containing Imipenem as Carbapenemase substrate Zinc for MBL producing gram negative bacilli. Test was interpreted as positive if colour changed from Red to Yellow or Red to Orange as shown in Figure 3 & Figure 4 respectively & Negative if no colour change observes From Red in reaction well e when compare to d control well as shown in figure 5 & reference provided in Table 1.

Figure 3: Positive Rapidec Carba NP test showing colour changed from Red in Control well d to Yellow in Reaction well e

Statistical Analysis: Statistical package of social science (SPSS) version 22.0 software was used for calculating various indices like sensitivity, of Rapidec Carba, NP test in detection of MBL producing Gram Negative Bacilli, & calculating occurrence of metallobetalactamase in paediatric patients.

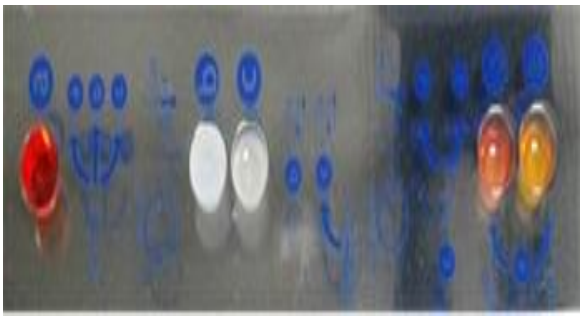

Figure 4: Positive Rapidec Carba NP test showing colour changed from orange in control well d to Yellow in Reaction well e.

Figure 5: Negative Rapidec Carba NP test with no colour change From Red in control well d to Red in reaction well e

Table 1: Interpretation of colour change in Control & Test well:

Control well	Test well	Interpretation
Red	red	Negative
Orange	orange	Negative
red	orange-red, orange to yellow	Positive
Orange	yellow	Positive
any colour other than red	-	Uninterpretable
Orange	red	Uninterpretable

RESULT

Out of Total fifty carbapenemase positive isolates, forty one were detected by Rapidec Carba Np test, giving sensitivity of 82 % for carbapenemase detection. Total twenty isolates (40%) were MBL positive. Out of twenty phenotypically characterised MBL positive isolates, eighteen isolates were detected correctly by Rapidec Carba Np test, with sensitivity of 90% for MBL class of carbapenemase, while two MBL positive isolates were failed to be detected by Rapidec Carba Np Test. Most commonly isolated spp in MBL positive Gram Negative Bacilli was Klebsiella pneumoniae (n=13), following by Acinetobacter spp (n=6), & one Roseomonas gilardii isolate. Among carbapenemase producing Gram Negative bacilli too, Klebsiella spp was commonest (n=26), followed by Acinetobacter spp (n=18), E.coli (n=2), Enterobacter cloacae (n=1), Citrobacter spp (n=1), Roseomonas gilardii (n=1), Pseudomonas spp (n=1).

Frequency of isolation of carbapenemase from different fluid was as follows, Blood (n=43), CSF (n=3), Pus (n=2), Urine (n=2).

Most of the carbapenemase positive gram negative bacilli isolated from blood were Klebsiella (n=25), CSF(n=1, Klebsiella spp, & n=2, Acinetobacter spp), Pus(n=1, Acinetobacter spp, n=1, Enterobacter cloacae), Urine (n=1, Acinetobacter , N=1, Citrobacter spp).

DISCUSSION

Sensitivity of Rapidec Carba NP test in present study was 90% in detecting MBL positive Gram Negative bacilli, which is comparable to Study conducted by Garg A et al who tested pre-characterised strains of New Delhi Metallo beta lactamase with this kit with good diagnostic accuracy. Sensitivity shown for carbapenemase detection is 82 %, which is low in comparison to manufacturers specifications of 97.8% sensitivity & several other studies conducted on this kit. [27-33] As Negative samples are not included in the study & molecular test was not performed this can be assume due to weak carbapenemase activity of class of Carbapenemase like OXA 48. [30,33] All MBL positive were detected in initial 5 to 10 minutes as also shown by Dortet et al. [30-33] another study from National Reference Centre for antibiotic resistant described 99% sensitivity. Poirel & Nordmann detected 5 false negative results in assessment study of Rapidec Carba NP test. Isolates were P.aeruginosa GES-2, A.baumanniiNDM-1, & OXA-23, Acinetobacter pittii producing OXA - 40, Proteus mirabilis producing chromosomally encoded OXA-23. Most common MBL positive Isolates were Klebsiella pneumoniae, followed by Acinetobacter spp & Roseomonas gilardii. Most common specimen was Blood then CSF & Sputum, Urine. [30-33]

Limitation of our study is negative isolates are not tested, so false positives couldn't be detected. But Poirel & Nordmann detected three false positive isolates from 75 non-carbapenemase gram negative bacilli. Overproduction of chromosomal Ampcs in Acinetobacter baumannii, & Proteus vulgaris positive for plasmid encoded CMy-2. Kabir et al also had shown false positive results in AMPC producing Enterobacter spp & Pseudomonas aeruginosa with OprD loss &up regulated efflux. [30-33]

But MBL positive gram negative bacilli detection is easy by Rapidec Carba Np test as it incorporates Zinc in reaction wells causing rapid detection of this class of carbapenemase which also explains rapid detection of MBL positive isolates, in initial 5 to 10 minutes as also shown by Dortet et al too. [30-33]

CONCLUSION

With 90% sensitivity & Turnaround time of 30 minutes to 2 hours, Rapidec Carba NP test proves effective screening test in adjunct with other phenotypic test. Forty percent carbapenem resistant isolates were MBL positive in Paediatric patients,

suggesting increased antibiotic resistance in paediatric patients too.

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