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.

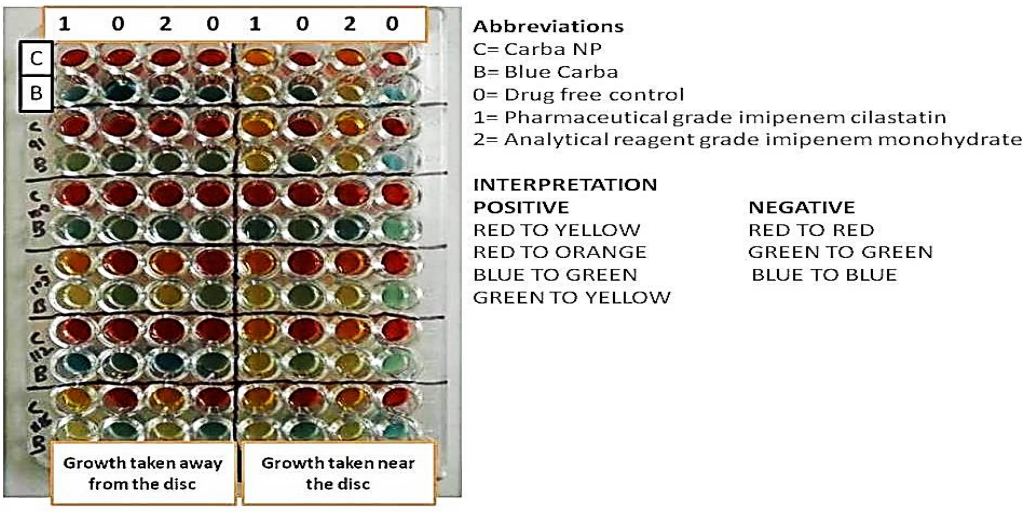

Fig. 1: Various tests with their variations

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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