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Detection of carbapenemase production in *Enterobacteriaceae* and *Pseudomonas* species by carbapenemase Nordmann–Poirel test

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Abstract:

PURPOSE: Multidrug-resistant organisms causing community-acquired and hospital-acquired infections are increasing at a dangerous rate. Carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas* species are an important source of concern since these organisms are not only resistant to beta-lactam antibiotics but also show cross-resistance to other groups of antibiotics. In the present study, rapid detection of these carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas* species by carbapenemase Nordmann–Poirel (Carba NP) test was evaluated by comparing with modified Hodge test (MHT).

MATERIALS AND METHODS: Imipenem-resistant *Enterobacteriaceae* and *Pseudomonas* species isolated from various samples such as pus, blood, sputum, urine, and endotracheal aspirates were processed for carbapenemase detection by MHT and Carba NP test. Kappa analysis was done to evaluate the percentage agreement between the two tests.

RESULTS: Seventy imipenem-resistant *Enterobacteriaceae* and *Pseudomonas* isolates were analyzed in the present study for carbapenemase production. 63.41% of *Enterobacteriaceae* and 34.48% of *Pseudomonas* species were carbapenemase producers considering both the methods. By MHT, 36 (51.42%) isolates and, by Carba NP test, 35 (50%) isolates were positive for carbapenemase production out of the 70 isolates.

CONCLUSION: Carba NP test when compared to MHT is a simple, rapid, cost-effective biochemical test which can be used in all laboratories in the identification of life-threatening carbapenemase-producing Gram-negative bacteria.

Key words:

Carbapenemase, carbapenemase Nordmann–Poirel, *Enterobacteriaceae*, modified Hodge test, *Pseudomonas*

Introduction

Multidrug-resistant organisms causing community-acquired and hospital-acquired infections are increasing at a dangerous rate globally, especially among *Enterobacteriaceae* and nonfermenters.^[1] Extended-spectrum β -lactamase (ESBL)- and acquired

cephalosporinase (AmpC)-producing organisms are resistant to almost all β -lactams with the exception of carbapenems. Treatment of choice for these ESBL- or AmpC-producing isolates is carbapenems.^[2] Therefore, it is important to preserve the clinical efficacy of carbapenems (imipenem, ertapenem, meropenem, and doripenem). However, in recent times, there is an increase in

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reporting of carbapenem-resistant *Enterobacteriaceae* worldwide, probably as a result of carbapenemase gene acquisition^[3] resulting in treatment failure due to carbapenem usage. Various mechanisms of carbapenem resistance in *Enterobacteriaceae* are due to a decrease in bacterial outer-membrane permeability, with excess production of β -lactamases with no carbapenemase activity or expression of carbapenemases.^[4,5] *Klebsiella pneumoniae* carbapenemase (Ambler Class A); Verona integron-encoded metallo- β -lactamase, imipenemase, and New Delhi metallo- β -lactamase (all Ambler Class B); and oxacillinase-48 (Ambler class D) are some examples of carbapenemases reported in *Enterobacteriaceae*.^[6]

Due to other resistance mechanisms, most of the times carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas* species show resistance to other groups of drugs also leading to multidrug-resistant or pandrug-resistant isolates. Rampant spread of such isolates is an important source of concern globally.^[7] This shows that for the selection of appropriate therapeutic schemes and the implementation of infection control measures, detection of carbapenemase producers is important.^[8,9] Rapid identification of carbapenemase is the need of today's clinical practice. Ultraviolet spectrophotometry, matrix-assisted laser desorption ionization-time of flight technique, and molecular methods are few examples of techniques available for rapid detection. Even though these methods have good sensitivity and specificity, they require trained microbiologists and expensive equipment. Molecular methods which are considered as gold standard method may fail to detect unknown carbapenemase genes not included in gene panel.^[10] To overcome all these drawbacks, a biochemical test (carbapenemase Nordmann-Poirel [Carba NP] test) based on a technique designed to identify the hydrolysis of the β -lactam ring of a carbapenem has been developed.^[11] The present study was undertaken to evaluate Carba NP test in discriminating carbapenemase producers from nonproducers by comparing with modified Hodge test (MHT).

Materials and Methods

This study was conducted in the department of microbiology of a teaching hospital. Ethical clearance certificate was obtained from the Institutional Ethical Committee. Various samples such as pus, blood, sputum, urine, and endotracheal aspirates received in the laboratory were inoculated on a sterility-checked MacConkey agar and blood agar plates and incubated at 37°C for 18–24 h. Based on the growth on MacConkey agar and blood agar, isolates were further processed in VITEK 2 systems, for identification and antimicrobial susceptibility. Imipenem-resistant *Enterobacteriaceae*

and *Pseudomonas* species were further processed for carbapenemase production by MHT and Carba NP test. Kappa analysis was done to evaluate the percentage agreement between MHT and Carba NP tests.

Modified Hodge test

Lawn culture of ATCC *Escherichia coli* 25922 at a turbidity equivalent to that of 0.5 McFarland was made onto the Mueller-Hinton agar plate. After drying, an imipenem (10 μ g) disc was placed at the center of the plate. The test strain and control strains (a known carbapenemase-producing *Pseudomonas aeruginosa* was used as positive control, and *E. coli* ATCC 25922 was used as negative control) were heavily streaked from the edge of the imipenem disc to the periphery of the plate in different directions. The plates were incubated at 37°C for 18–24 h.

Interpretation

The presence of a cloverleaf type of zone of inhibition near the test/positive control organism was interpreted as MHT positive.^[12]

Carbapenemase Nordmann-Poirel test

The Carba NP test for *Enterobacteriaceae* and *Pseudomonas* spp. was performed as follows:

Two 1.5-ml low-bind protein microcentrifuge tubes (Eppendorf), each containing 100 μ l of a 20-mM Tris-HCL lysis buffer, were individually inoculated with a 1- μ l loopful of bacterial colony (18–24 h old, loop swept through pure culture), and bacterial suspensions were vortexed for 5 min. To the first tube, 100 μ l of 0.5% (wt/vol) phenol red solution with 10-mM zinc sulfate (solution A, buffered to pH 7.8 by adding 0.1 N NaOH) was then added, and the tube was vortexed. To the second tube, 100 μ l of solution A with imipenem dissolved directly in solution A to a final concentration of 6 mg/ml was added and then vortexed. A mixture of the phenol red solution and the enzymatic suspension being tested was incubated at 37°C for a maximum of 2 h.^[13] A known carbapenemase-producing *P. aeruginosa* was used as positive control, and *E. coli* ATCC 25922 was used as negative control.

After incubation, the presence of any carbapenemase, which hydrolyzes imipenem into its carboxylic form, leading to a pH decrease, was detected by a color change of phenol red solution (red to yellow/orange), while tubes remain red in the absence of carbapenemase.

Interpretation

1. First tube and second tube remaining red – noncarbapenemase-producing isolate
2. First tube remaining red and second tube turning yellow/orange – carbapenemase-producing isolate.

Results

Seventy imipenem-resistant *Enterobacteriaceae* (19 – *Klebsiella* species, 15 – *E. coli*, 3 – *Enterobacter cloacae*, 2 – *Morganella morganii*, and 2 – *Serratia* species) and *Pseudomonas* species (29) isolates were analyzed in the present study.

Out of 29 *Pseudomonas* species, 14 were from pus samples followed by 12 from endotracheal aspirates. Out 19 *Klebsiella* species, 8 were from pus and 7 from urine. Sample-wise distribution of isolates is shown in Table 1.

Out of 29 imipenem-resistant *Pseudomonas* species studied, 17 (58.6%) isolates were resistant to meropenem and 18 (62.0%) isolates were resistant to doripenem. Twenty-seven (93.1%) isolates were found to be sensitive to colistin.

Of the 19 *Klebsiella* species studied, total resistance was observed to imipenem and meropenem. Sixteen (84.2%) isolates were found resistant to ertapenem. Thirteen (68.4%) isolates were resistant to tigecycline and 15 (78.94%) isolates were sensitive to colistin. Of the 15 *E. coli* studied, total resistance was observed to imipenem and meropenem. Ten (71.4%) were sensitive to tigecycline and 13 (92.8%) isolates were found to be sensitive to colistin.

Out of 70 isolates analyzed for carbapenemase production, 36 (51.42%) isolates were carbapenemase producers by MHT and 35 (50%) isolates were detected as carbapenemase producers by Carba NP test. Out of 29 *pseudomonas* species, 10 (34.48%) isolates were detected as carbapenemase producer by MHT. Out of these 10 isolates, one isolate gave negative result for carbapenemase production by Carba NP test.

Among 41 *Enterobacteriaceae*, 26 (63.41%) isolates were detected as carbapenemase producers. Out of 19 *Klebsiella* species, 14 (73.68%) and, out of 15 *E. coli*, 10 (66.66%) were detected as carbapenemase producers by both MHT and Carba NP test, respectively. One *Morganella* out of 2 and 1 *Enterobacter* species out of 3 were carbapenemase producers by both the methods. Both *Serratia* species were negative for carbapenemase production by both the methods. Kappa analysis

revealed that the strength of agreement between the two tests is considered very good (kappa = 97%, confidence interval = 0.916–1.00).

Discussion

One of the greatest advances of modern medicine is the development of antibiotics for the treatment of infectious disease. Unfortunately, effectiveness of many antimicrobial agents is under threat due to the emergence of antibiotic resistance among bacteria. In order to control the emergence of drug resistance, the irrational use of antibiotics should be controlled. One of the ways of controlling antibiotic misuse is rapid detection and reporting of drug resistance mechanisms in clinical isolates, which helps in the selection of appropriate antibiotics for treatment. Keeping this in mind, the present study evaluated Carba NP test for rapid detection of carbapenemase among *Enterobacteriaceae* and *Pseudomonas* species.

A total of 70 clinical isolates (29 *Pseudomonas* and 41 *Enterobacteriaceae*) were analyzed for carbapenemase production. 63.41% of *Enterobacteriaceae* were carbapenemase producers (66.66% of *E. coli* and 73.68% of *Klebsiella* species). Chauhan *et al.*^[14] reported 87.01% of *E. coli* and 91.51% of *Klebsiella* spp. as carbapenemase producers by MHT. These findings suggest that carbapenemase production is on rise in *Enterobacteriaceae*, particularly in *Klebsiella* species. 34.48% *Pseudomonas* species were detected as carbapenemase producers, and similar findings were observed in a study done by ElMasry *et al.*,^[15] in which 37% of *Pseudomonas* species were reported as carbapenemase producers by polymerase chain reaction. However, by MHT, positivity was increased to 48.1%.

One *Pseudomonas aeruginosa* strain in the present study was positive for carbapenemase production by MHT but negative by Carba NP test. Several studies have shown that GES-type carbapenemase-producing *Pseudomonas* species may not be detected by Carba NP test.^[16] This could be one of the reasons in the present study for Carba NP test showing negative results. However, in this study, molecular analysis was not performed to comment on sensitivity or specificity of Carba NP test.

In the present study, MHT test was performed using imipenem disc instead of ertapenem or meropenem disc because we were able to reproduce and interpret results better using imipenem disc than meropenem. Even though both Carba NP and MHT detected carbapenemase producers almost equally except for one strain which gave negative results with Carba NP test, MHT at times was difficult to interpret.

Table 1: Sample-wise distribution of clinical isolates

Organism	Pus	Endotracheal aspirates	Urine	Blood	Sputum
<i>Pseudomonas</i> species (29)	14	12	0	0	3
<i>Klebsiella</i> species (19)	5	10	2	2	0
<i>Escherichia coli</i> (15)	8	0	7	0	0
<i>Enterobacter cloacae</i> (3)	2	0	0	1	0
<i>Serratia marcescens</i> (2)	0	2	0	0	0
<i>Morganella morganii</i> (2)	2	0	0	0	0

Conclusion

Carbapenemase producing Gram-negative bacteria causing community-acquired and hospital-acquired infections are increasing at a dangerous rate globally. Methods for rapid identification of these organisms is of utmost importance. One such method is Carba NP test. When compared to MHT, Carba NP test is a simple, rapid, cost-effective biochemical test which can be used in all laboratories in the identification of life-threatening carbapenemase-producing Gram-negative bacteria.

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Conflicts of interest

There are no conflicts of interest.

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