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Original Article

Evaluation of the compatibility of phenotypic and molecular methods used to determine carbapenem resistance in *enterobacterales* isolates

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Abstract

Aim: Carbapenems are one of the most important options for clinicians with few treatment options in the clinic due to their low side effects, rapid diffusion into tissues and use in all age groups. Therefore, it is important to be able to detect carbapenemase-producing isolates at an early stage for appropriate patient management and for infection prevention and control procedures. Antibiotic resistance genes and enzymes of microorganisms can be determined by phenotypic and molecular methods in clinical microbiology laboratories. Phenotypic methods are cheap, easy and easy to repeat but determination of resistance gene regions by molecular methods is costly, requires labour-experienced personnel and is time consuming. Determining whether the isolates possess the carbapenemase enzyme by phenotypic tests will provide convenience both for the patient and early initiation of the treatment and directing the clinician to the treatment. In this study, we aimed to evaluate the compatibility of phenotypic methods (carbapenemase inactivation method and Rapidec Carba NP) and molecular methods (Polymerase Chain Reaction) used to determine carbapenem resistance in *Enterobacterales* isolates.

Material and Methods: Carbapenem resistant 60 and sensitive 20 *Enterobacterales* isolates were included in the study. E-test agar gradient diffusion, CIM, Rapidec Carba NP methods and PCR were studied. The agreement between the methods was determined by using the kappa (κ) coefficient with the cohen kappa analysis method.

Results: In carbapenem resistant isolates, meropenem MİK50 and MİK90 determined as 32µg/ml, 64µg/ml, imipenem MİK50 and MİK90 determined as 32µg/ml, 128µg/ml, respectively. OXA-48 was positive in 54 (90%) isolates and NDM-1 in 6 (10%) isolates. The susceptibility of the isolates with OXA-48 carbapenemase gene region was 94.4% by CIM test and 92.6% by Rapidec Carba NP test, respectively. When the Kappa coefficient was evaluated, a very good agreement was observed between both tests and OXA-48. However, in the isolates with NDM-1 gene region, no compliance with CIM test was observed but Rapidec Carba NP test showed very good agreement.

Conclusion: Rapid carbapenemase testing, such as Rapidec Carba NP and CIM, can play an important role in preventing the development of health-related outbreaks caused by carbapenemase-producing isolates, enabling faster prevention and control of infection.

Keywords: *Enterobacterales*; carbapenem; resistance; rapidec carba np; carbapenemase inactivation method

INTRODUCTION

Enterobacterales constitutes the most frequently isolated gram negative bacteria group among the hospital and community-acquired infectious agents (1). In recent years, supportive treatments, invasive interventions and irrational and long-term antibiotic treatment in hospitals have facilitated the spread of antibiotic resistance among these isolates and the selection of strains which multiple drug resistance. The treatment option of these isolates is extremely limited (1,2).

Carbapenem group antimicrobials are indispensable agents in the treatment of various life-threatening infections caused by expended spectrum and AmpC β-lactamase-producing isolates, due to their low side effects, good tissue distribution, and no age restrictions. In addition, carbapenems can be used safely to treat the multidrug-resistant bacterial infections in adults and children (3). However, increasing the global incidence of carbapenem resistant Enterobacterales phenotypes with a rapid acceleration every day causes difficulties in the regulation and management of patients'

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treatment regimens (3,4). Therefore, this causes prolonged hospitalization and increase the mortality rates of infected patients (4). The U.S. Centers for Disease Control and Prevention (CDC) estimates that carbapenem resistant isolates are responsible for 9300 (6.6%) of approximately 140,000 healthcare-associated Enterobacterales infections in the U.S. each year (5).

Active surveillance studies have been carried out in intensive care units of our hospital since 2006. As a result of these studies, in the Enterobacterales strains isolated from intensive care units, imipenem resistance was 4% and meropenem resistance was 5% in 2012. However, in 2015, we determined that imipenem resistance rate increased to 35% and meropenem resistance rate to 42% (6). Therefore, it is extremely important to detect the carbapenemase-producing isolates at an early stage for proper patient management and implementation of infection prevention and control procedures.

Molecular detection of genes encoding carbapenemase is the gold standard for demonstrating carbapenemase resistance. However, daily and practical application of molecular methods in most clinical laboratories is limited. In addition, molecular techniques have some disadvantages such as high cost and important expertise requirement (7). Therefore, simple, reliable and low-cost methods are required for screening microorganisms that produce carbapenemase for infection control measures. In this study, we aimed to compare the results of the Polymerase Chain Reaction (PZR), carbapenemase inactivation method (CIM) and Rapidec Carba NP method results in the isolates which were resistant to one or both meropenem and imipenem.

MATERIAL AND METHODS

Working group

Sixty carbapenem resistant and 20 carbapenem sensitive Enterobacterales isolated from clinical samples sent to our laboratory between December 2017 and July 2018 were included in the study. Isolates were selected which has not clonal relationship and their identification was done by conventional methods and Malditoff MS (BioMérieux, France).

E-test agar gradient diffusion

In order to determine carbapenem resistance, imipenem and meropenem minimum inhibitory concentration (MIC) values were studied by E-test agar gradient diffusion method. For this purpose, the ready-made E-test package was removed from the freezer (-20 °C) 30 minutes before. The turbidity of the isolate colony to be tested was made as 0.5 McFarland emulsion. A sterile cotton swab was dipped in the inoculum and it was applied to Mueller-Hinton agar. E tests were placed on agar surface and plates were incubated at 37 °C for 18-24 hours. The MIC value was read as the point at which the ellipse cuts the scale.

Carbapenemase Inactivation Method (CIM)

Four-hundred µl saline was added to the eppendorf tube. A small amount of bacteria was taken and mixed with this saline. The meropenem (10µg) disc was added to the mixture. Its mouth was closed and kept in 37 oC for 4 hours. The meropenem disc, which was kept for 4 hours, as well as an untreated meropenem disc (for control purposes) were placed on the Mueller-Hinton agar medium with 0.5 McFarland density E. coli ATCC (25922). It was kept in 37 oC for 1 night with the condition of being at least 6 hours.

While evaluating the results, the sample with no inhibition zone around the meropenem disc was considered to be carbapenemase positive, and the samples with the inhibition zone as carbapenemase negative.

Rapidec Carba NP Test

Ready-to-use Rapidec Carba NP test kits (BioMérieux, France) were used. The test was interpreted by working according to the manufacturer's instructions. Any color change from red to yellow or red to orange between the control well and the test, which can be seen with the naked eye, was accepted as a positive test result.

Polymerase Chain Reaction (PCR)

Carbapenem resistance genes were investigated by polymerase chain reaction. Open system Palm Cycler PCR device was used for PCR method. Internal controls were used to detect PCR inhibition during the study and external controls were used to control DNA isolation. Carbapenem resistance genes were studied as suggested by Poirel et al. (8). Carbapenemase resistance gene regions was investigated using primers, OXA–48-F GCGTGGTTAAGGATGAACAC OXA–48-R CATCAAGTTCAACCCAACCG for blaOXA-48, NDM–1-F GGTTTGGCGATCTGGTTTTC, NDM-1-R CGGAATGGCTCATCACGATC for blaNDM-1, KPC-F-TCGCTAAACTCGAACAGG and KPC-R- TTACTGCCCGTTGACGCCCAATCC for blaKPC, için VIM-F-GATGGTGTTTGGTCGCATA, VIM-R- CGAATGCGCAGCACCAG for blaVIM, IMP-F-GGAATAGAGTGGCTTAAYTCTC and IMP-R-CCAAACYACTASGTTATCT for blaIMP.

Statistical analysis

The agreement between the methods was determined by using the kappa (κ) coefficient with the Cohen kappa analysis method. As the significance level was 0.05, κ = 0.61-0.80 good level of fit, κ = 0.81-1.00 very good level of fit (9). Analyzes were made using IBM SPSS Statistics for Windows version 25.0 (NY, USA).

RESULTS

Sixty carbapenem resistant and 20 carbapenem sensitive isolates were included to the study. 59 of the carbapenem resistant isolates were K. pneumoniae (98.3%) and 1 was E. coli (1.7%), 10 of carbapenem sensitive isolates were K. pneumoniae and 10 were E. coli. OXA-48 was positive

in 54 (90%) isolates and NDM-1 was positive in 6 (10%) isolates. All of the isolates identified as carbapenem resistant were imipenem and meropenem resistant. Meropenem MIK50 was determined as 32 µg / ml, MIK90 was 64 µg / ml, imipenem MIK50 was 32 µg / ml, MIK90 was 128 µg / ml.

In 54 isolates which have OXA-48 gene region, carbapenemase positivity was determined as 50 (92.6%) with Rapidec Carba NP test and 51 (94.4%) with CIM test. While the Rapidec Carba NP test was detected as positive in all isolates which have NDM-1 gene region, but CIM test was found positive as only 1 (16.7%) isolates (Table 1). When Cohen's kappa analyzes was examined, OXA-48 gene region showed a very good agreement with the isolates determined with both tests. However, in isolates with NDM-1 gene region, Rapidec Carba NP test showed very good compatibility, while CIM test did not. In the negative controls, no positivity was detected with both tests.

Table 1. Compatibility of the methods

Please use dot instead of comma	Positive n(%)	Kappa	p
OXA-48/ Rapidec Carba NP(n=54)	50(92.6%)	0.871	<0.001
OXA-48/ CIM (n=54)	51(94.4%)	0.902	<0.001
NDM-1/ Rapidec Carba NP (n=6)	6 (100%)	1.000	<0.001
NDM-1/ CIM (n=6)	1(16.7%)	0.20	0.182

Negative control K. pneumoniae(n=10)
Negative control E. coli (n=10)
κ=0.61-0.80 good compatibility, κ=0.81-1.00 very good compatibility (9) Please use dot instead of comma

DISCUSSION

Rapid increase of resistance rates in Enterobacterales isolates, which is one of the most important pathogens causing health care associated infections, has become a global public health concern (1). The widespread use of antibiotics facilitates the emergence of multidrug-resistant Enterobacterales group bacteria by conjugation of resistance genes between the bacterial genus (1,2). Over the past decade, the prevalence of carbapenem-resistant Enterobacterales-related bacterial infections has increased worldwide.

Irrational and long-term antibiotic treatment in hospitals facilitates the spread of antibiotic resistance and cause selection of multiple drug resistance strains, especially in Enterobacterales (6). Multiple drug resistance of these isolates, limits the treatment options and leads to high morbidity and mortality. In various studies, it is reported that mortality due to Enterobacterales bacteria in intensive care units is between 30-54% (10). Increasing resistance to antibiotics such as carbapenem and colistin, which are used as the last group of antimicrobials in infections due to multi-drug resistant Enterobacterales isolates, is a clinically important concern. Moreover, there are increasing publications reporting that these isolates are not only limited in hospitals but also spread to the public (11). In a prospective multicenter study involving 260 infected or colonized patients with carbapenem resistant K. pneumonia, found that 39% of patients with bloodstream infections or pneumonia died and their length of hospital stay was 5-10 days (12).

Carbapenems are broad-spectrum antimicrobials that are considered the last-line treatment for bacterial infection with multiple drug resistance. Carbapenems are the most important treatment options for clinicians due to their rapid diffusion into tissues, low side effects than colistin and tigecycline and used in all age groups (3). Therefore, it is important to be able to detect carbapenemase-producing isolates at an early stage for proper patient management and implementation of infection prevention and control procedures. Carbapenem resistance of these bacteria is due to the production of metallo-lactamases such as IMP, NDM and VIM-type enzymes, KPC-type-ß-lactamases and acquired carbapenemases such as OXA type. OXA-48-like carbapenemases are among the most common mechanisms in Europe, the Middle East and South America (1, 7, 13).

In clinical microbiology laboratories, resistance genes and enzymes of microorganisms against antibiotics can be determined by phenotypic and molecular methods. Detection of resistance gene regions by PCR, which is one of the molecular methods, is accepted as the gold standard (8). However, PCR requires costly, time consuming, serious workforce, experienced personnel. Therefore, cheap, easy and easy to repeat phenotypic methods are generally preferred in clinical microbiology laboratories. Demonstration of the carbapenemase enzyme in isolates by phenotypic tests will provide convenience to clinicians in the treatment of patients.

Carbapenemase inactivation method is an important phenotypic test that has a low cost, can be applied easily in routine laboratories and give results quickly. Also, an inhibitor-based biochemical assay "Carbapenemase Nordmann-Poirel" (Carba NP) test for carbapenemase detection, is recommended by the "European Committee on Antimicrobial Susceptibility Testing (EUCAST)" (14). We detected the carbapenemase gene in Enterobacterales isolates by using PCR molecular method. And the phenotypic methods CIM and Rapidec Carba NP tests were compare with the molecular method PCR to evaluate compatibility. If we examine the studies on this subject in the world, Kabir et al. (15) reported that the sensitivity of the Rapidec Carba NP test was 96.3% in isolates producing OXA-48-like carbapenemase and 100% in isolates producing NDM. Also Davarcı et al. (16) reported

that the sensitivity of CIM was 96.9%, Bayramoglu et al. (17) reported as 93.9%. Hombach et al. (18) reported Rapidec Carba NP sensitivity as 90.2% and specificity as 100% in their study on 252 isolates containing various carbapenemase genes. Aktaş et al. (19) reported that the sensitivity of Rapidec Carba NP test is 99% and CIM test it is 78% in 2 hours' incubation and 90% in 4 hours' incubation. Tijet et al. (20) reported that Rapidec Carba NP sensitivity was 90.1% and negative predictive value was 88.2%, and, CIM sensitivity was 98.8%, negative predictive value was 99%, respectively. The results obtained from the studies Yamada et al. (21), Osterblad et al. (22), Dortet et al. (23) show that the sensitivity of CIM is 97.1%, specificity is 95.7% for Enterobacterales. Also these studies reported the specificity of the Carba NP test is 100%, sensitivity varies between 72.5-100% (21-23). In our study, the sensitivity of isolates containing OXA-48 carbapenemase gene region was determined as 94.4% with CIM test and 92.6% with Rapidec Carba NP test. When the kappa coefficient was evaluated, a very good agreement was observed between both tests and OXA-48. However, in isolates which have NDM-1 gene region, Rapidec Carba NP test showed very good compatibility, while CIM test did not. These results were similar to those previously reported except NDM-1 gene region and CIM test compatibility. This may be due to the low number of Enterobacterales isolates which has NDM-1 gene region.

CONCLUSION

In summary, both methods showed high sensitivity and specificity in detecting OXA-48 carbapenemase, which is the most common in our country. Rapidec Carba NP test is simple and fast that takes 2.5 hours for the detection of carbapenemase production, and the CIM is a simple and low cost that takes 24 hours and does not require special equipment. Rapid carbapenemase tests such as Rapidec Carba NP and CIM can play an important role in preventing health-related outbreaks caused by carbapenemase-producing isolates by providing infection prevention and faster implementation of control measures.

Conflict of interest: The authors declare that they have no competing interest.

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