Current fast methods for the identification of carbapenemase enzymes in clinical laboratory

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ABSTRACT:

Introduction: Multidrug resistance is growing at an alarming rate among a variety of bacterial species, causing infections, which threaten the lives of patients. Detection of metal-β-lactamase producers (IMP, VIM, NDM) and of KPC producers may be based on the inhibitory properties of several molecules but requires additional expertise and time. Several authors describe the use of new rapid identification methods. The aim of our review is to revise the new rapid methods for the detection of carbapenemases and their identification by Gram-negative bacilli.

Keywords: Carbapenemases, Multi-drug resistence, CRE, KPC detection, Carbapenems, Gram negative bacteria.

INTRODUCTION

Nowadays, one of the most important threats in medicine, and especially in infectious diseases, is the emerging resistance to extended-spectrum β-lactams of Gram-negative bacilli. Multidrug resistance is growing at an alarming rate among a variety of bacterial species, causing infections which threaten the lives of patients[1-9]. Among the resistance mechanisms characterizing these organisms, the production of various β-lactamase enzymes is the basis for their acquired resistance to different antibiotics. Treatment regimens for many hospital- and community-acquired infections are constituted primarily by β-lactam drug class; however, these antimicrobials are rapidly becoming obsolete, due to the rapid spread of β-lactamases among Gram negative bacilli[8,10-16]. As a matter of fact, the β-lactamase-operated hydrolysis of β-lactam antibiotics is their most common mechanism of resistance[17-25]. Currently, β-lactamases are classified according to two systems: a molecular classification, based on the amino acid sequence, and a functional and structural classification scheme proposed by Bush et al[1,2] in 1995, which takes into account substrate and inhibitor profiles. The molecular classification is simpler and worldwide accepted. It differentiates β-lactamases in four molecular classes: A, B, C, and D. Classes A, C, and D include enzymes that, through an active serine site, hydrolyze their substrates by forming an acyl-enzyme, whereas class B β-lactamases are metalloenzymes promoting β-lactam hydrolysis through utilize at least one zinc-ion active-site.

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Class A carbapenemase group includes members of the infamous SME, IMI, NMC, GES, and KPC families.

Conversely, class B enzymes exhibit a broad spectrum of hydrolytic activity, including penicillin, cephalosporins, and carbapenems. Their activity only spares the monobactam aztreonam[3,21,25,26]. These enzymes possess the greatest carbapenemase activity, and they are mostly of the IMP, VIM and NDM types. Class B β-lactamases are not inhibited by commercially available β-lactamase inhibitors (clavulanic acid, tazobactam, or sulbactam). Avibactam is an exception to this rule, except for VIM β-lactamases. Class B enzymes-mediated hydrolysis depends on the interaction of the β-lactam with zinc-ion in their active site, and is inhibited by ehylendiaminetetraacetic acid (EDTA), a chelator of divalent cations, and dipicolinic acid.

Ambler class C cephalosporinases, such as AmpC and CMY-2, and ESBLs, such as CTX-M-15 and SHV-2, confer resistance to a majority of the available β-lactams. Reduced susceptibility to carbapenems in most organisms was mostly attributed to a combination of production of extended-spectrum β-lactamase or AmpC β-lactamase and deficiency of porins in the outer membrane[27,28].

Class D enzymes showing carbapenemase activity are mostly OXA-48[4]. An increasing number of Ambler class A carbapenemases (such KPC enzymes), class B metallo-β-lactamases (VIM, IMP, and NDM β-lactamases), and class D carbapenemases like OXA-23, OXA-48 have recently emerged[4].

The biggest concern is the emergence and worldwide spread of carbapenemases such as KPC, OXA-48, VIM, IMP, New Delhi metallo-β-lactamase-1 (NDM-1) among the Enterobacteriaceae. These carbapenemases confer to bacteria the potential to virtually resist to all β-lactams[20,29,30]. In Europe, a mixture of KPC, NDM, VIM, and OXA-48-like carbapenemases dominates, while IMP-producing bacteria are more prevalent in the South-East countries[31-37]. Detection of metallo-β-lactamase producers (IMP, VIM, NDM) and of KPC producers may be based on the inhibitory properties of several molecules, but requires additional expertise and time, as it usually requires near 24-48 hours. However, several authors describe the use of new rapid identification methods.

The aim of this paper is to revise these new rapid methods for the detection of carbapenemases and their identification by Gram-negative rods.

OVERVIEW

KPC

Resistances developed because of *Klebsiella pneumoniae* carbapenemases (KPCs) were first identified in 1996 in a *K. pneumoniae* isolate obtained from a patient hospitalized in North Carolina, USA. KPCs are plasmid-encoded enzymes capable of hydrolysing a broad spectrum of beta-lactams, including carbapenems and monobactams. The first outbreak of KPC-producing *K. pneumoniae* outside the United States was described in Israel in 2006[38-42]. Bacteria characterized by the *Klebsiella pneumoniae* carbapenemase (*bla*-KPC) gene have caused outbreaks globally with substantial mortality and morbidity. KPC enzymes are typically encoded within the Tn3-based transposon Tn4401, which has five known isoforms named from “a” to “e”, as defined by insertions or deletions within a polymorphic region immediately upstream of *bla*-KPC[43-45]. Members of the *K. pneumoniae* ST258 lineage usually remain susceptible to colistin, gentamicin and tigecycline, but there have been documented outbreaks of colistin-resistant *K. pneumoniae* ST258[46]. About 11 different KPC variants (from KPC1/2 to KPC12) have been described; however, in Italy only KPC-2 and KPC-3 have been reported, with a higher prevalence of KPC-3[46].

OXA

Class D β-lactamases, also known as OXA-type enzymes or oxacillinases, are represented by more than 350 genetically diverse enzymes across Gram-negative rods. First identified enzymes were penicillinases, which hydrolyzed oxacillin, conferring resistance to this antimicrobial agent, hence the name oxacillinases and the prefix OXA. These enzymes are of clinical interest, due to their ability to produce resistance to carbapenems, the last resort in the treatment of severe infections, such as sepsis and intra-abdominal infections[4,47]. OXA β-lactamases were among the earliest β-lactamases. Subsequently, the plasmid-encoded β-lactamases (OXA-23, OXA-40, and OXA-58) were categorized as OXA enzymes, because of their structural similarity to earlier OXA β-lactamases. The OXA-23 β-lactamase was first identified in an *A. baumannii* isolate with a 16 MIC for Imipenem, with a transferable resistance phenotype, indicating a plasmid location[47-52]. There is a rapid emergence of oxacillinase (OXA-48) that increasing frequency respect to the classical carbapenemases (KPC, NDM, IMP, and VIM) across the world.

VIM

Verona integron-encoded metallo-β-lactamase (VIM) and Imipenemase (IMP) belong to group B of Ambler classification. They cause hydrolysis of a variety of beta-lactam antimicrobials except for monobactam (aztreonam). These enzymes are unique, because their activity is inhibited by chelating agents such as Ethylene Diamine Tetra Acetic Acid (EDTA), Sodium Mercapto Acetic Acid (SMA), and Dipicolinic Acid, but do not result inhibited by beta-lactamases inhibitors such as clavulanic acid, sulbactam, tazobactam and avibactam. VIM-1 was firstly identified in *Pseudomonas aeruginosa* in 1999. Currently, twenty-four of the 46 VIM variants have been identified in *Pseudomonas aeruginosa*[53-56]. Initially observed in *Pseudomonas aeruginosa* and *Acinetobacter* spp, these enzymes spread to members of the family *Enterobacteriaceae* during early 2000s and they have been involved in different outbreaks, in particular in Italy and Greece[35,53].

NDM

New Delhi metallo-beta-lactamase (NDM) have been report first time in 2009 in urine cultures resulted positive to *Klebsiella pneumoniae*. Subsequently, it has spread globally causing various types of infections[1,3,47,57]. The organisms that carry genetic material for NDM have been found to be characterized by a great ability of transferring genes encoding NDM to other organisms with immense power of spread. The NDM-1 gene (bla_{NDM-1}) is normally carried on a variety of plasmids along with other resistance factors, and may confer resistance to all β-lactams molecules[20,24,30,58,59].

Commercial Test

The detection of carbapenemases in clinical microbiology labs is time- and human-resource- consuming because phenotypic tests like triple-disc test (Rosco Diagnostics, Denmark) may be difficult to read. Matrix-assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS) and Polymerase Chain Reaction (PCR)-based molecular methods have good sensitivity and specificity, but trained personnel and good infrastructure are required. Moreover, these tests are very expensive. Classical molecular methods are considered the gold standard for detection of carbapenemases-encoding genes. However, these methods require specific equipment, supplies, expertise, and a routinely use of these tests may not be affordable by all laboratories because of the costs.

NEW MOLECULAR METHODS

XPERT CARBA-R

Xpert® Carba-R is performed on Gene Xpert system. It is fully automated and integrated for sample preparation, nucleic acid extraction, amplification and detection of targets using Real-time PCR (RT-PCR) assays. The Xpert Carba-R Assay includes reagents for the detection of *blaKPC*, *blaNDM*, *blaVIM*, *blaOXA*-48, and *blaIMP* gene sequences, associated with carbapenem-non-susceptibility in Gram negative bacteria as well as a sample processing control (SPC) that contains spores of *Bacillus globigii* in the form of a dry bead that is included in each cartridge to verify for adequate processing of the target bacteria and for indicate the presence of inhibitors in the PCR reaction. Xpert® Carba-R test is built on the basis of real time PCR and it is simple to perform with the minimal hands-on time of 15 min and a short Turn Around Time (TAT) of < 1 hour[60]. The results are interpreted by the GeneXpert System from measured fluorescent signals and shown in the view results window. The Xpert® Carba-R kit is currently the only commercially available assay that can also detect IMP-1 group producer. Xpert® Carba-R kit may be used directly on rectal swabs such that offers an efficient option for the rapid screening of carbapenemase producing organisms (CPO) for colonized patients. Cortegiani et al[60] used Xpert® Carba-R for rapid detection of carbapenemase-producing bacteria in abdominal septic patients admitted to intensive care units (ASPA-ICU) and show that the sensitivity detection was 50% and specificity was 93.1% (positive predictive value (PPV) 80%; negative predictive value (NPV) 77.1%). The test may be considered an additional diagnostic tool for early diagnosis of carbapenem resistance in abdominal septic patients (B). The Xpert® Carba-R v2 (now covering OXA-181 and OXA- 232) presents a 100% sensitivity, 99.13% specificity, 85.71% positive predictive value and 100% negative predictive value. Hoyos-Mallecot et al [80] demonstrated that the Xpert® Carba-R v2 kit is well adapted for rapid screening of high-risk patients even in low prevalence regions (in <1 h versus 24/48 h for culture)[60-62]. The cost per cartridge is about $ 54.6 per test.

Check-Direct CPE for BD MAX™

Check-Direct CPE (Becton, Dickinson and Company, NJ, USA) is an automated Real-time (RT)-PCR for detection of the *Enterobacteriaceae* that made a carbapenemase enzyme clinically prevalent such IMP, VIM, NDM and OXA gene by routine suspected isolates. The samples, bacterial cell suspension, must to be processed within 72 hours according to the manufacturer's instructions. The instrument automatically performs sample lysis, DNA extraction, multiplex RT-PCR, and reports results in less than 3 hours. Several studies show a good sensitivity and specificity in the detection of carbapenemases genes also from rectal swabs compared with cultural methods[63-65].

COLORIMETRIC METHODS

RAPIDEC® CARBA NP

BioMérieux has recently introduced the Rapidec® Carba NP test kit for rapid detection of carbapenemase-producing Gram-negative bacteria. The Rapidec® Carba NP test is based on the direct detection of Carbapenem hydrolysis by Carbapenemase-producing bacteria, providing rapid results within a time ranging from 30 minutes and two hours. The Rapidec® Carba NP test showed excellent specificity and sensitivity, allowing reliable detection of known carbapenemases in *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. It showed both a sensitivity and specificity of 96%[66]. Rapidec® Carba NP test showed excellent performance with all bacterial species and none carbapenemase activity was detected among *A. baumannii* strains resistant due to the overexpression of their intrinsic chromosomally encoded enzyme like OXA-66. This test is performed by isolated on culture plant-agar (requiring 24 or 48 hours of incubation); an inoculum of colonies taken from a culture plate is mixed with reagents. Eye reading of the plate is made between 30 min and 2 hours of incubation at 37°C.

A test is deemed as positive when there is a variation of color from red either to yellow or to orange. Several papers showed that the Rapidec® Carba NP was simple and relatively inexpensive, the cost is only $5 per test furthermore the test is easy to perform and read and, in most cases, results are available after <30 min[66-72].

β-*CARBA*™

β-CARBA™ (Bio-Rad, Marnes-la-Coquette, France) is a commercial rapid qualitative colorimetric test used for detecting strains with a decreased susceptibility to carbapenems due to the production of carbapenemases. Several studies report that the test shows both sensitivity and specificity between 97 and 100%. The duration of this test is extremely short, with a hands-on time of less than 3 min, and it is performed directly on isolated colonies on agar plates (time needed for cultures 24-48 h). One loop of colony material is resuspended in the kit solution inside of microtubes, vortexed and incubated at 35°C. The reading of the reaction is performed after 30 min, but a re-evaluation of the test could be needed at additional timepoints. However, a prolonged incubation time leads to a decrease in specificity. A color change from yellow to red or orange indicates a positive result[73,74].

Neo-Rapid Carb Test

Neo-Rapid Carb test (Rosco) is similar in operation to the Carba NP test, but it works with tablets containing imipenem as indicator and negative-control tablets. Moreover, it does not require laboratory manipulation of antibiotics. The test procedure consisted of adding several loops of the tested strain to a mixture of 100 μl 0.9% NaCl and 100 μl of extraction buffer for made at suspension of 4 McFarland. This suspension was then vortexed and incubated at room temperature for at least 30 min; 50 μl was transferred to a tube containing 100 μl of 0.9% NaCl. Subsequently, a tablet of imipenem plus indicator was added, and the tube closed and vortexed for disintegrate the tablet and then incubated at 35 to 37°C between 30 min to 2 h. A positive result was interpreted as a color change from pink or red to yellow or orange-yellow. Sometimes the results are not easy to be read. Several studies show that this method exhibited a high level of accuracy, with 96% sensitivity and 100% specificity. The cost is approximatively 6$ per test[75,76].

IMMUNOCHROMATOGRAPHIC TEST

Coris Bioconcept

Coris BioConcept (Coris BioConcept, Gembloux, Belgium) has launched a first immunochromatographic rapid diagnostic test for *in vitro* identification of carbapenemases in bacteria culture. The KPC *K*-SeT® assay is a diagnostic test, based on fast immunochromatography, able to rapidly detect KPC-type carbapenemases from bacterial cultures. The test using colloidal gold nanoparticles bound to nitrocellulose membrane sensitized with monoclonal antibodies has been developed for KPC detection directly from a single bacterial colony. The test is able to detect KPC-2, KPC-3 and KPC-4 within 3 min after inoculation of the cassette, other KPC types are detect in different time after inoculation of cassette but ever within few minutes. This assay presented high predictive positive and negative values comprise between 100% and 92.3%, respectively. The Coris BioConcept made also RESIST-3 O.K.N. K-SeT for detection of KPC, OXA-48, NDM enzymes and RESIST-3 O.O.K for detection of OXA-48, OXA-163, KPC and RESIST-4 O.K.N.V for revelation of OXA-48, KPC, NDM, VIM[77-81]. **Table 1** resumes rapid identification techniques and the enzymes identified.

Table 1. Rapid identification tests of carbapenemases and enzymes identified.

TEST	Productors	Enzymes or Gene detected	Cost		References
Xpert® Carba-R	Cepheid	*blaKPC, blaNDM, blaVIM, blaOXA, and blaIMP*	$54.6 per test	Swabs or coltures	(60), (61), (62)
Check-Direct CPE for BD MAX™	Becton Dickinson	*blaKPC, blaOXA, blaVIM, blaNDM*	N/A	Swabs or coltures	(63), (64), (65)
Rapidec® Carba NP	bioMerieux	KPC, NDM-1, VIM, IMP, OXA	$5 per test	Coltures	(66), (67), (68), (69), (70), (71), (72)
β-CARBA™	Bio-Rad	KPC, NDM, VIM, IMP, SPM, OXA	N/A	Coltures	(73), (74)
Neo-Rapid CARB Test	Rosco	KPC, NDM, IMP, VIM, OXA	$6 per test	Coltures	(75), (76)
NEW-Resist	*Coris Bioconcept*	KPC, NDM, VIM, OXA	N/A	Coltures	(77), (78), (79), (80), (81)

CONCLUSIONS

Systems able to detect and identify carbapenemases described above are, nowadays, of the utmost importance. These are now well adapted to the European epidemiology of CPE, which possess a number of mechanisms of resistance. The production of β-lactamase enzymes confers to Gram-negative bacteria the biggest part of the acquired resistance to different class of the antibiotics. According to the manufacturers' indications, the investigation of an outbreak may efficiently reduce the impact, duration and cost of patients' hospitalization.

Conflict of Interest:
The Authors declare that they have no conflict of interests.

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