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Review article

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Carbapenemase Detection and Identification: Which method should be Chosen?

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Abstract

Carbapenemase production constitutes the key mechanism of resistance to carbapenems. Rapid detection or confirmation of the involvement of carbapenemase-producing bacteria (CPB) in infections is crucial for adequate antibiotic therapy and infection control, particularly during epidemics or for surveillance purposes. The Ambler classification proposes four classes of carbapenemases: classes A, B, C, and D. Carbapenemases A, C, and D are called serine carbapenemases because they use serine in their active site to catalyze the hydrolysis of carbapenems. Class B carbapenemases are metallo-beta-lactamases (MBLs) that use a cation (Zn2+) to hydrolyze the beta-lactam ring. Biochemical, chromogenic, immunochromatographic, mass spectrometric, and molecular methods have both advantages and limitations for carbapenemase detection. Although there is no single method that meets all specifications of an ideal test, it is important to explore methods to identify the most suitable ones. Thus, according to the research articles reported in this review and the criteria (cost, turnaround time, sensitivity, specificity, expertise needs, target carbapenemases), chromogenic tests such as ChromID CARBA SMART and CHROMagar™ mSuperCARBA™ would be the best candidates for the rapid and effective detection of carbapenemase-producing bacteria in developing countries. Moreover, Carba NP, SUPERCARBA and Carbapenem Inactivation Method (CIM) could also be considered good carbapenemase-detecting methods. WGS may be reserved for large-scale funded studies of carbapenemase-producing bacteria.

Keywords: CarbapenemaseDetection Methods, Carbapenemase-Producing Bacteria, Carbapenemase

1. Introduction

Carbapenems constitute a class of antibiotics of last resort for the treatment of severe infections caused by multi-resistant or ultra-resistant bacteria [1,2].Carbapenems are bactericidal antibiotics that penetrate the cell wall of bacteria, bind to multiple penicillin-binding proteins (PBPs), and inactivate intracellular autolytic inhibitor enzymes, ultimately killing the bacterial cell [3,4]. Unfortunately, bacterial clones resistant to carbapenems are becoming widespread worldwide, with a prevalence of up to 50% in certain regions[5-9]. The World Health Organization (WHO) has reported carbapenem-resistant *Enterobacteriaceae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa* as critical priority pathogens for human health [10]. Three major mechanisms lead to carbapenem resistance: (i) production of carbapenemases (enzymes that hydrolyze carbapenems), (ii) overexpression of efflux pumps, and (iii) quantitative and/or qualitative loss of outer membrane porins (omp) associated with the production of extended-spectrum β-lactamases (ESBL) or cephalosporinases[4,11,12]. The production of carbapenemase constitutes the major mechanism of resistance to carbapenems [13,14].

CRB infections are often fatal with a mortality rate of up to 57%[15,16], Therefore, it is essential to rapidly detect or confirm the involvement of (CPB) in infections for adequate antibiotic therapy and infection control, especially during epidemics or for surveillance purposes. Phenotypic and genotypic methods are used to detect CPB. Thus, this review aimed to compare CPB detection methods and identify the best carabapenemase detection methods for use in developing countries.

2. Classification of Carbapenemases

There are many classifications for carbapenemases, but the two main classifications most commonly used are structural classification according to Ambler [17] and functional classification by groups based on substrate spectra according to Bush-Jacoby-Meideros[18,19]. Other known classifications have been proposed by Sawai, based on substrate profiles[20], and Richmond and Sykes, based on the functional characteristics of beta-lactamases[21].The Ambler classification proposes four classes of carbapenemases: classes A, B, C, and D. Carbapenemases A, C, and D are called serine carbapenemases because they use serine in their active site to catalyze the hydrolysis of carbapenems. Class B carbapenemases are metallo-beta-lactamases (MBLs) that use a cation (Zn2+) to hydrolyze the beta-lactam ring[17,22]. Thus, class A includes

(KPC, NMC, SFC, SME, IMI, and carbapenemases of the TEM, SHV, and CTX-M types)[23–25]. Class B includes MBLs (IMP, VIM, NDM, GIM, SIM, KHM, TMB, SPM, Bla2, DIM, BlaB, CcrA, BcII, CphA, Sfh-1, ImiS, FEZ-1, BJP-1, AIM -1, THIN-B, GOB-1, CAU-1, CAR-1, SMB, POM-1, and CRB11[26–28]. Class C includes variants of (CMY, ADC, PDC, FOX, MIR, ACT, GC1, TRU-1, and DHA) capable of hydrolyzing carbapenems[26]. Class D includes variants of oxacillinases (OXA) that are capable of hydrolyzing carbapenems.

Regarding the Bush-Jacoby-Medeiros classification, carbapenemases are found in group 1 (carbapenemase types ACT, CMY, ADC)[29]; subgroup 2be (CTX-M type carbapenemase, SHV-type carbapenemase, GES)[30,31]; subgroup 2df (OXA-48, OXA-23); subgroup 2f (KPC, SME, NMC-A, IMI, SFC)[19,25,32]; subgroup 3a (IMP, VIM, NDM, SPM, DIM, GIM, SIM, FIM, CcrA, IND, L1, FEZ-1, BJP-1, AIM-1, THIN-B, GOB-1, CAU-1 , CAR-1, SMB, POM-1 and CRB11)[19,26,32] and subgroup 3b (CphA, Sfh-I and ImiS) [19].

3. Carbapenemase Detection and Identification Methods

3.1. Modified Hodge Test

Also called the clover leaf method or MHT in abbreviation, its principle is the inactivation of a carbapenem by a strain (the strain tested) producing carbapenemase allowing a reference strain sensitive to carbapenems (*E. coli* ATCC® 25922) to extend its growth towards the carbapenem disk with the inoculum streak of the tested strain (Figure 1). A positive test result indicates an indentation similar to that of a clover leaf. MHT makes it possible to detect carbapenemase production but does not precise the carbapenemase involved. MHT has sensitivity (90-100%) and specificity (82.9-91%). This test has excellent sensitivity for detecting class A and D carbapenemases and has the advantage of being simple to perform and inexpensive[33,34]. However, this test sometimes yields false positives for strains hyperproducing cephalosporinases (AmpC) and for strains producing ESBL (CTX-M) associated with an alteration of porins [35]. In addition, it is difficult to identify strains that are weak carbapenemase producers[14,33,35–37]. MHT also presents numerous false negatives for the detection of MBL in general and NDM in particular (approximately 50% sensitivity for detection of MBL)[38]. Indeed, MHT has difficult to detect the activity of MBLs because they are anchored to the outer membrane of Gram-negative bacteria[39,40]. The addition of Triton X-100 (a synthetic detergent used in cell and molecular biology to permeabilize cytoplasmic membranes) to MHT agar enables the detachment of MBLs bound to the outer membrane, resulting in an MBL detection sensitivity of up to 90%. This variant of MHT is known as the Triton Hodge test (THT)[41].

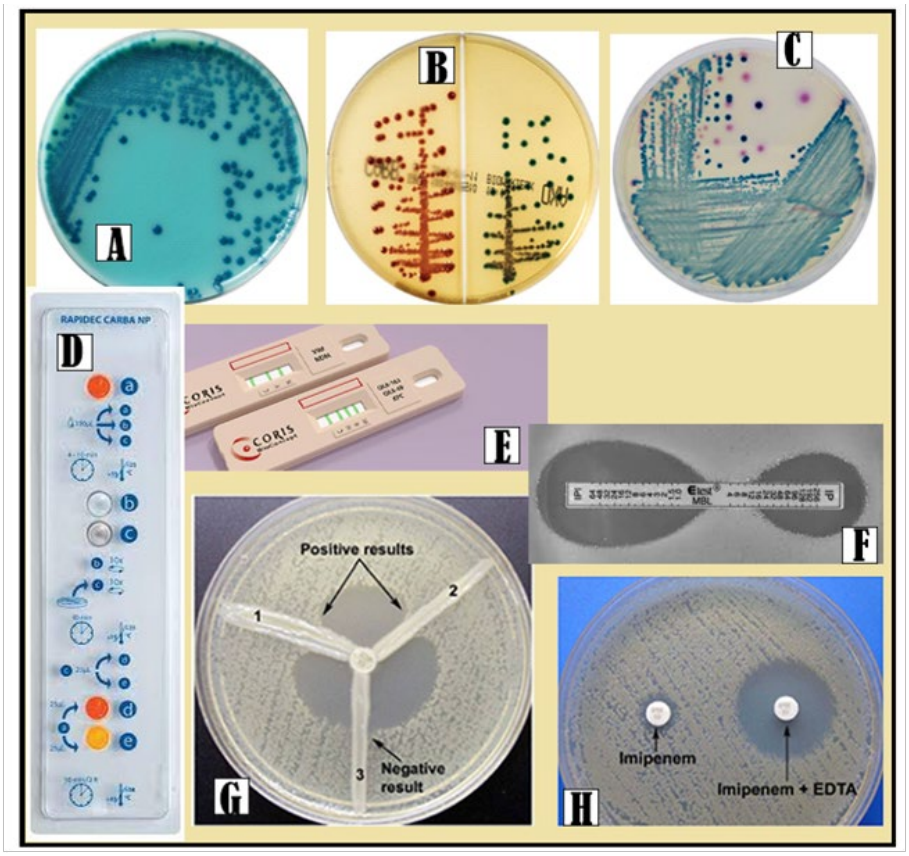

Figure 1: A, Brillance CRE agar ; B, CHROMID® CARBA SMART ; C, CHROMagar™ mSuperCARBA™; D, Rapidec Carba NP; E, RESIST-4 O.K.N.V. immunochromatographic test; F, MBL E-test; G, Modified Hodge test; H, combined imipenem and imipenem-EDTA disc test.

3.2. The Carbapenemase Inactivation Method (CIM)

The carbapenemase inactivation method (CIM) was introduced by the CLSI (2016). In this method, 400 µl of the tested bacterial suspension (10µL inoculation loop from a 1-day-old culture + 400 µL water) is incubated with a meropenem disk (10 µg) for 2 h. If the isolate produces carbapenemases, the meropenem present in the disc will be degraded. The disk is then removed from the broth and placed on Mueller Hinton agar, on which a meropenem sensitive reference strain of *E. coli* ATCC 29522 (0.5 McFarland) was inoculated. After 18-24 hours of incubation at 35°C, if the inhibition diameter is < 19 mm, the tested strain is concluded to be a carbapenemase producer [42,43]. This method has shown high concordance with the results obtained by PCR testing and is used in many clinical and public health laboratories. CIM and its variants have shown sensitivity (90.4-100%) and specificity (66.7-100%)[43-45]. CIM is easy to perform at a low cost; however, the type of carbapenemase involved cannot be identified. CIM does not produce optimal results for class B carbapenemases and false positives have been reported for strains hyperproducing AmpC-type cephalosporinases[44,45].

3.3. Detection of MBL Using Ethylene Diamine Tetra Acetic Acid

MBLs require zinc ions for carbapenemase activity. The principle of this test is to seek synergy between chelators such as EDTA and carbapenems. Various formats of this principle are used, the most common of which are disks (double disk synergy test or combined disk test) and MBL E-test strips (Figure 1). According to EUCAST recommendations, the detection of MBL in *Enterobacteriaceae* should be based on the combined meropenem and meropenem-EDTA disc tests. The test is considered positive when there is an increase in the inhibition zone of 5 mm or more[14]. The disk method should be interpreted with caution and monitoring the intrinsic activity of EDTA is strongly recommended. The MBL E-test combines carbapenem alone on one side of the strip and carbapenem-EDTA on the other. The differential observed in terms of MIC allows for the possible demonstration of MBL production. The MBL detection method, based on the synergy between EDTA and carbapenem, has a sensitivity of (86.2-100%) and specificity of (43.1-100%)[46–49]. Carrying out these tests is easy, inexpensive, and does not require special equipment but lasts for at least 24 h (the incubation time of the test). These tests enable the identification of class B carbapenemase production without specifying the family of MBLs involved. Certain specificity problems with certain strains, particularly *Acinetobacter baumannii*, have been raised. In addition, interpretation problems occur when the MIC of carbapenem is low. An example of a test based on the synergy between EDTA and carbapenems is the "Etest® MBL MP/MPI," marketed by Biomérieux[33,50,51].

3.4. Detection of Carbapenemases by Chromogenic Culture Media

Several selective chromogenic culture media have been developed to directly isolate carbapenem-resistant strains by producing carbapenemases from almost all types of samples, between 18h-24h of culture. They consist of a rich nutritional base, combined with patented antibiotic blends. These chromogenic media are very affordable, do not require special equipment or knowledge, are very fast, and contain chromogenic molecules that make it possible to recognize *Enterobacteriaceae* species. They have a sensitivity and specificity superior to 75%. Chromogenic culture media can isolate carbapenemase-producing bacteria using direct sample or fresh bacterial culture. The best-known brands of chromogenic culture media are CHROMagar KPC, mSuperCARBA™, ChromID CARBA SMART, ChromID CARBA, Brilliance™ CRE Agar, and SUPERCARBA[52-54] (Figure 1).

3.4.1. CHROMagar KPC

CHROMagar KPC, marketed by CHROMagarTM, makes it possible to excellently identify Gram-negative bacilli producing KPC and having a carbapenem MIC >16µg/ml, but is less sensitive for strains with low resistance to carbapenems. CHROMagar KPC also makes it possible to identify strains resistant to ertapenem and sensitive to other carbapenems. CHROMagar KPC has sensitivity (75.4-100%) and specificity (92.7-100%)[55-58].

3.4.2. CHROMagar™ mSuperCARBA™

CHROMagarTM also markets CHROMagar™ mSuperCARBA™, which makes it possible to effectively isolate *Enterobacteriaceae* and non-fermentative bacilli (*Acinetobacter* spp. and *Pseudomonas* spp.) that produce KPC, VIM, GIM, NDM, and OXA-48[54,59]. CHROMagar™ mSuperCARBA™ has a sensitivity (93.1-100%) and specificity (96.2-100%)[54,59,60].

3.4.3. ChromID CARBA and ChromID CARBA SMART

ChromID CARBA and ChromID CARBA SMART are marketed by BioMérieux. ChromID CARBA SMART has the advantage of enabling the isolation of the most widespread carbapenemases, such as OXA-48, KPC, and even NDM-type MBLs with a sensitivity and specificity of respectively 86.7% and 100%[45]. ChromID CARBA has a sensitivity (85.5-100 %) and specificity (87.5-100%)[52,54,56,61-63]. ChromID® CARBA agar is reported to be not suitable for the detection of CPE with slightly increased minimum inhibitory concentrations (MIC) against carbapenems[64].

3.4.4. Brilliance™ CRE Agar

Brilliance™ CRE agar, marketed by Thermo Fisher Scientific (Waltham, Massachusetts, USA), enables the isolation of Gram-negative bacilli producing KPC, VIM, GIM, NDM, and OXA-48. Brilliance™ CRE agar has the advantage of allowing the isolation of strains with low levels of resistance to carbapenems and has a sensitivity for KPC, VIM, GIM, and NDM ranging from 57 to 100%. However, a weakness of this medium is that its specificity is reduced ranging from 43.3-100% for all classes of carbapenemases (due to the cultivation of strains producing AmpC and/or ESBL on Brilliance™ CRE agar).Brilliance™ CRE agar has decreased sensitivity for OXA-48 and MBLs[54,62,63,65-68].

3.4.5. Supercarba

SUPERCARBA is a selective chromogenic medium based on Drigalski agar, zinc sulfate, carbapenem, and cloxacillin. It was the first chromogenic medium to isolate *Enterobacteriaceae*, producing large families of carbapenemases, such as KPC, NDM, OXA-48, IMP, and VIM. SUPERCARBA also enables the detection of strains that are low producers of carbapenemases. It has sensitivity (80-100%) and specificity (52.2-98.5%) [56,65,67-69].

3.5. RESIST-4 O.K.N.V. Immunochromatographic Test

A multiplex immunochromatographic test, RESIST-4 O.K.N.V. (Figure 1) uses monoclonal antibodies to rapidly detect OXA-48 variants, KPC, NDM, and VIM carbapenemases. This assay has (54.2-100%) sensitivity and (91.8-100%) specificity and can be performed using pure bacterial colonies or direct samples. The immunological assays depend on the level of carbapenemase production. This assay can be completed in 24H and has the advantage to precise the involved carbapenemase but cannot detect other carbapenemases apart from KPC, VIM, NDM, and OXA-48. This assay is rapid, inexpensive, and does not require strong expertise [70-73].

3.6. Carba NP Method and Variants

This is a method for biochemical detection of carbapenemases in *Enterobacteriaceae, Pseudomonas* aeruginosa, and *Acinetobacter baumannii* in less than two hours. Its principle is based on *in vitro* hydrolysis of the β-lactam ring of imipenem by the strain tested, producing a carboxylic derivative that decreases the pH, leading to a change in the color of the phenol red indicator (red to yellow/orange) (Figure 1). Carba NP test is inexpensive, rapid (within 2 h), requires no special equipment, and detects all known Ambler class A, B, and D carbapenemases with sensitivity (68.2-100%) and specificity (92-100%). The Carba NP test can be performed on bacterial colonies and direct samples such as blood cultures [33,43,74-77]. In addition to the aforementioned advantages, the Carba NP test perfectly differentiates carbapenemase-producing strains from carbapenem-resistant strains due to mechanisms not mediated by carbapenemases, such as combined resistance mechanisms (defects in permeability of the outer membrane associated with overproduction of cephalosporinase and/or ESBL). The Carba NP test also perfectly differentiates carbapenemase-producing strains from strains sensitive to carbapenems, but expresses a broad-spectrum beta-lactamase without carbapenemase activity (ESBL, hyperproduction of AmpC-type cephalosporinases). Despite its advantages, the Carba NP test cannot differentiate between carbapenemase classes and does not detect carbapenemases with low carbapenemase activity, such as GES. It is also associated with a very low number of false negatives, particularly in mucoid strains. Despite these limitations, the Carba NP test is excellent for detecting carbapenemases. The "RAPIDEC® CARBA NP" marketed by bioMérieux and the "Rapid CARB Screen" marketed by ROSCO Diagnostic are two marketed tests derived from the Carba NP test[69,78,79].

3.7. Detection of Cabapenemase Production by MALDI-TOF Mass Spectrometry

Its principle is based on the search for a modification of the spectrum of a carbapenem under the effect of carbapenemase (produced by the strain tested). In this test, a fresh bacterial culture is mixed with a carbapenem solution and the mixture is incubated for three hours. The mass spectra of the intact molecule and its degradation products are analyzed. This technique has a sensitivity (76-100%) and specificity (95.3-100%) for the detection of class A, B and D carbapenemases, but with a reduced sensitivity for the detection of class D carbapenemases, such as OXA-48. It has been shown that the addition of NH4HCO3 to the reaction buffer increases the sensitivity of OXA-48 detection by MALDI-TOF MS from 76% to 98% [80-85]. A variant of the MADI-TOF MS method was developed with a reduced analysis time from 4 h to 30 min with direct reading from Petri dishes[81]. Since 2018, it has been possible to use an improved version of the MALDI-TOF-MS technique to detect the production of carbapenemases directly from bottles of positive blood cultures [82].The detection of carbapenemase production by MALDI-TOF mass spectrophotometry does not make it possible to specify the type of carbapenemase produced, and false positives sometimes occur in CTX-M-type ESBL-producing bacteria. This technique requires fine-tuning, particularly trained personnel, and a mass spectrometer (a very expensive device)[84,86].

3.8. Detection of Carbapenemase Genes by Molecular Methods

Molecular methods are the reference methods, even if the detection of a carbapenemase gene does not necessarily indicate its expression or does not confer on the detected gene full responsibility for this resistance. Molecular techniques make it possible to precisely identify carbapenemase gene(s) and their variants, and also have epidemiological significance. The best-known and used techniques are the search for carbapenemase genes by end-point PCR, real-time PCR, and bacterial whole-genome sequencing.The analysis time ranges from 2 h to a few days, depending on the method chosen. Some of these methods can only be carried out after the extraction of bacterial DNA, but others can be perfomed directly on bacterial colonies or directly from samples (stool, rectal swabs, and others). However, molecular techniques have high production costs and require specialized equipment and advanced knowledge. This means that these methods have limited access in developing countries. Furthermore, apart from whole-genome sequencing, the range of carbapenemase genes detected is predefined by the panel of primers used. This makes undetectable new carbapenemases whose primers have not been used[53,87].

3.8.1. Detection of Carbapenemase Genes by End-Point PCR

PCR was developed in the 1980s [88]and allows rapid and exponential amplification of target DNA sequences using specific primers (sense and antisense) and DNA polymerase in the presence of deoxyribonucleotides. There are three steps: denaturation of the double-stranded DNA, hybridization, and final elongation. It can be performed in simplex (search for a single gene at a time) or

multiplex (search for several genes simultaneously). The amplicons resulting from PCR are migrated and observed under ultraviolet light in the presence of intercalating agents. The entire process, from amplification to visualization, can take between 4 and 5 h. Endpoint PCR has the advantage of determining the expected size of the amplification products. The detection of carbapenemase using end-point PCR has sensitivity (96-100%) and specificity (97-100%) [33,89–91]. Table 1 shows primers sequences for the detection of key carbapenemase genes.

Genes	Primers sequences	Amplicon size (bp)	Hybridization temperature(°C)	Reference
bla_{NDM}	F: 5' - GGTTTGGCGATCTGGTTTTC - 3' R: 5' - CGGAATGGCTCATCACGATC - 3'	621	52	[50]
bla_{OXA-48}	F: 5'- TTGGTGGCATCGATTATCGG - 3' R: 5'- ATGGAACCCACATCGACATT - 3'	743	60	[92]
bla_{OXA-23}	F: 5' - TCTGGTTGTACGGTTCAGCA - 3' R: 5' - GCAAAAGCGACAATTTTTCC - 3'	501	55	[93]
bla_{VIM}	F: 5' - GTTTGGTCGCATATCGCAAC - 3' R: 5' - AATGCGCAGCACCAGGATAG - 3'	382	55	[94]
bla_{KPC}	F: 5' – CTGTCTTGTCTCTCATGGCC – 3' R: 5' – CCTCGCTGTGCTTGTCATCC - 3'	636	62	[95]
bla_{IMP}	F:R'- GAAGGCGTTTATGTTCATAC– 3' F:R'- GTACGTTTCAAGAGTGATGC– 3'	587	60	[91]

Table 1: primers sequences for the detection of key carbapenemase genes

3.8.2. Detection of Carbapenemase genes by RT-PCR

RT-PCR allows the amplification and detection of the target carbapenemase gene in a single step, with considerable time savings (some results can be obtained in less than an hour). RT-PCR uses fluorescent probes that undergo conformational reorganization when they bind to their targets and emit fluorescence. Fluorescence intensity is directly proportional to the quantity of amplicons. RT-PCR is also characterized by its high selectivity, which enables the detection of the target carbapenemase gene from a sample containing other DNA molecules (host cells, contaminants). Different methods for detecting amplicons are possible: either non-specific, such as SYBR Green (BioRad), or specific, such as TaqMan (BioRad), Molecular Beacons or FRET (fluorescence resonance energy transfer) probes [96–98]. The detection of carbapenemase using RT PCR has sensitivity (97-100%) and specificity (100%)[99–101].One test based on RT-PCR methods is the Xpert Carba-R real-time PCR-based assay.

3.8.3. Xpert Carba-R real-time PCR-based assay

The Xpert Carba-R test is a qualitative real-time PCR-based assay that detects and differentiates the most prevalent carbapenemase gene families within 48 min (KPC, NDM, VIM, IMP-1, OXA-48, OXA-181, and OXA-232). This assay can be performed on direct samples such as blood, sputum, and rectal swabs) without the need for culturing. Xpert Carba-R by rapidly identifying (< 1 hour) patients colonized by carbapenemase-producing bacteria can contribute to rapid patient care to avoid the occurrence and spread of carbapenemase-producing bacteria (CPB) epidemics. This assay has a sensitivity (93.5-100%) and specificity (94.2-99%). Xpert Carba-R would therefore constitute a good tool for monitoring, and controlling CPB. This test and its devices are expensive and require qualified personnel[102–109].

3.8.4. Detection of Carbapenemase Genes by whole Genome Sequencing

New sequencing techniques (NTS) include second-(SGS) and third-generation (TGS) sequencing techniques, enable the study of bacterial genomes and have the advantage of simultaneously detecting the sequences of known carbapenemase genes, sequences of new carbapenemase genes, mobile genetic elements (EGM) carrying these genes, and phylogenetic information[87]. In addition, the reduction in sequencing costs and increase in available genomic sequences have favored the creation of databases and web tools that are freely accessible on the Internet, thus allowing rapid genomic analysis. Whole-genome sequencing requires the intervention of highly trained personnel, and sequencing devices are very expensive.Overall, WGS remains the gold standard method for investigating carbapenemase-producing bacteria with sensitivity (96.3-100%) and specificity (> 99%). The limitation of WGS is that it cannot accurately demonstrate that a specific carbapenemase gene is expressed phenotypically in bacteria [110–113].

Methods	Sensitivity	Specificity	Specimen type	Turn-around time	Cost (incuding equipment)	Expertise needs	Target carbapenemase	limitation	Gene identification	IRMOCP	Ref
Modified Hodge test (MHT)Ɣ	90-100%	82.9-91%	fresh bacterial culture	18-24H	Very cheap	Low expertise	class A, B#, D	- False Positif for AmpC and ESBL producers with porins alteration - difficulty to identify weak carbapenemase producers - does not provide information regarding the type of carbapenemase involved	No	No	[14,33, 35–37]
Carbapenem inactivation methods (CIM) and variants	90.4-100%	66.7-100%	fresh bacterial culture	18-24H	Very cheap	Low expertise	class A, B#, D	- False Positif for AmpC producers - does not provide information regarding the type of carbapenemase involved	No	No	[42–45]
Detection of MBL using EDTA (EDTA Combined-disc test and EDTA-based E-test)	86.2-100%	43.1-100%	fresh bacterial culture	18-24H	Very cheap	Low expertise	Class B (MBL)	- The family of MBL is not specified - for EDTA-based E-test, interpretation problems occur when carbapenemase MIC is low	No	No	[14,33, 46–51]
CHROMagar KPC	75.4-100%	92.7-100%	Direct sample, fresh bacterial culture	18-24H	cheap	Low expertise	KPC	- difficulty to identify weak carbapenemase producers - only KPC-producing bacteria are detected	No	No	[55–58]
CHRO-Magar™ mSuperCARBA™	93.1-100%	96.2-100%					KPC, MBL, OXA-48	Not reported	No	No	[54,59 ,60]
ChromID CARBA SMART	86.7	100%					OXA-48, KPC, NDM	Not reported	No	No	[45]
ChromID CARBA	85.5-100 %	87.5-100%					class A, B, D#	difficulty to identify weak carbapenemase producers	No	No	[54,56, 61–64]
Brilliance™ CRE agar	57-100%	43.3-100%**					class A, B#, D#	- False Positif for AmpC and ESBL producers with porins alteration - Reduced sensitivity for class B and D carbapenemases	No	No	[54,62, 63,65–68]
SUPERCARBA	80-100%	52.2-98.5%**					class A, B, D	Not reported	No	No	[56,65, 67–69]
RESIST-4 O.K.N.V. immunochromatographic test	54.2-100%	91.8-100%	fresh bacterial culture, direct sample	1-24H	cheap	Low expertise	class A#, B, D	Can only detect KPC, NDM, VIM and OXA-48-type carbapenemase	No	No	[70–73]

Carba NP test and variants	68.2-100%	92-100%	Bacterial colony, direct sample	< 2H	cheap	Low expertise	class A, B#, D#	- difficulty to identify weak carbapenemase producers	No	No	[33,43,74–77]
MALDI-TOF mass spectrometry	76*-100%	95.3-100%	fresh bacterial culture, positive blood cultures	30min - 4H	Very expensive	Requires expertise	class A, B, D#	- The family of carbapenemase is not specified - False Positif for CTX-M producers - Reduced sensitivity for class B and D carbapenemases	No	No	[80–85]
RT-PCR	97-100%	100%	DNA	3-5H	expensive	Requires expertise	depending on the primers used.	- Detect carbapenemases according to primers used	Yes	No	[33,99–101]
Xpert Carba-R	93.5-100%	94.1-99.4%	fresh bacterial culture, DNA, Direct sample	48 min	expensive	Requires expertise	KPC, NDM, VIM, IMP-1, OXA-48, OXA-181, and OXA-232	Others carbapenemases cannot be detected	Yes	No	[102–109]
End-point PCR	96-100%	97-100%	fresh bacterial culture, DNA, Direct sample	4-5H	expensive	Requires expertise	depending on the primers used.	- Detect carbapenemases according to primers used	Yes	No	[33,89–91]
WGS	96.3-100%	> 99%	DNA	Few days	Very expensive	Requires strong expertise	All carbapenemases	Not reported	Yes	Yes	[87,110, 111,113]

IRMOCP, Identification of resistance mechanisms other than carbapenemase production; GNB, Gram negative bacteria; the addition of NH4HCO3 to the reaction buffer increases the sensitivity of class D detection by MALDI-TOF MS from 76% to 98%; WGS, Whole genome sequencing; MBL, metallo-beta-lactamase; #, reduced sensitivity, specificity or problem of detection; **, reduced specificity for non-fermenting bacteria; Ɣ, addition of zinc sulphate or Triton X-100 improve the MHT performance;

Table 2 :summary of carbapenemase detection and identification methods

4. Conclusion

Prompt management of CPB-associated infections is crucial to prevent fatal outcomes. CPB outbreaks often occur in hospitals. Therefore, it is appropriate to systematically test high-risk patients and quickly obtain accurate results during or before the admission procedure for better care of patients and their hospitalization environment, such as beds.High-risk patients are: from the intensive care units, patients with postoperative complications, hospitalized elderly and newborn patients, immunocompromised patients, and patients hospitalized in the oncology and hemo-oncology departments. These detection methods must be chosen according to their cost, rapid time to obtain results, sensitivity, and specificity. Although there is no single test that meets all specifications of the ideal test, as described in this review, chromogenic tests such as ChromID CARBA SMART and CHROMagar™ mSuperCARBA™ would be best candidates for inclusion in protocols for the rapid and effective detection of carbapenemase-producing bacteria in developing countries. Carba NP, SUPERCARBA and CIM could also be considered as good methods. WGS may be reserved for large-scale funded studies of carbapenemase-producing bacteria. Moreover, most carbapenemase detection methods do not detect class C carbapenemases. Thus, many cases of class C carbapenemases will go unnoticed. Future carbapenemase detection methods may address this issue.

Conflict of interest

The authors declare that they have no conflict of interest.

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