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Carbapenemases in Enterobacteriales: identification and screening

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Acquired resistance to carbapenems_is a global problem facilitated by plasmid-mediated spread of class A (KPC), B (IMP, VIM, NDM) and D (OXA-48-like) β-lactamases among multiple species, including *Escherichia coli*, *Klebsiella*, *Enterobacter*, and *Citrobacter* species. There is an increasing number of hospital outbreaks due to those bacteria which are also resistant to many other families of antibiotics. These outbreaks are difficult to control and overlap with spread to and within the community. Whereas KPC producers remain mostly hospital-acquired *K. pneumoniae*, spread of VIM, NDM and OXA-48-like producers is now observed in hospital and community-acquired infections since those latter carbapenemase genes are located in a variety of enterobacterial species such as in *E. coli*. Although globalization of carbapenemase producers in ongoing, endemicity of KPC producers in known in particular in the USA, Italy, Greece, Portugal and India, NDM producers in North Africa, Middle East, Eastern Europe, Asia, and OXA-48-like producers in North Africa, Africa and India.

In Switzerland, we are witnessing to the isolation of an increasing number of carbapenemase producers. Therefore rapid and accurate detection of carbapenemase-producing Enterobacteriales (CPE) is becoming fundamental to any strategy aiming at controlling their diffusion as well as for guiding the antibiotherapy. Indeed, the transferability of these genes explains largely their rapid spread. In addition, whereas KPC and OXA-48 producers remain almost always susceptible to novel combinations of β -lactam/ β -lactam inhibitors such as ceftazidime-avibactam, this is not the case for IMP/VIM/NDM producers which activities are not inhibited by avibactam.

Many methods are used to identify CPE including phenotypic susceptibility testing, selective culture media, immunochromatographic assays, specific PCRs and sequenced-based molecular tests. All techniques display variable degrees of sensitivity and specificity. CPE may be identified in two clinical situations, either infection or colonization.

A- Infection

Testing shall be performed from isolated colonies:

- 1- Identification.
 - MALDI-TOF
 - $\circ~$ or at the species level: Vitek (bioMérieux) or Phoenix (PMIC/ID Panels) for example
- 2- Susceptibility testing.

Carbapenems	MIC breakpoints (mg/L)		Disk content (µg)	Zone diameter breakpoints (mm)	
	S≤	R >		S ≥	R <
<u>Ertapenem</u>	0.5	0.5	10	25	25
<u>Imipenem</u>	2	4	10	22	17
Imipenem, Morganella morganii, Proteus spp. and Providencia spp.	0.125	4	10	50	17
Meropenem	2	8	10	22	16
Meropenem-vaborbactam	8	8			

EUCAST 2019 guidelines v.9.0

- Note that *Proteus* sp. and *Morganella* sp. are naturally less susceptible to carbapenems as compared to other enterobacteriales. For carbapenemase screening, a meropenem screening cut-off >0.12 mg/L (zone diameter <28 mm) is recommended
- Varbobactam at a concentration of 8 mg/L

• Inhibition tests based on addition of dipicolonic acid and EDTA to carbapenem containing disks or E-test strips may contribute to differentiate class B carbapenemases from class A and class D. They are specific and sensitive but need an additional 24 h of culture.

• In addition, it shall be mentioned that OXA-48-type producers are resistant to temocillin (although temocillin-resistant bacteria may be associated to other mechanisms). Therefore, this resistance marker can be useful for recognition of OXA-48-like producers, even though it is not specific only to OXA-48-like enzymes, with for example class B carbapenemase producers also exhibiting high-level resistance to that molecule.

3- Detection of carbapenemase activity may be performed by use of biochemical and immunological techniques

• A biochemical detection of carbapenemases may be based on the rapid detection of imipenem hydrolysis (Rapid Carba NP test: Rapidec Carba NP test, bioMérieux, Geneva (Fig. 1)) (CLSI/EUCAST guidelines, 2019). Results are obtained mostly in 30 min-1 h with good specificity and sensitivity. It detects any kind of carbapenemase activity. A few OXA-48-like strains produce that carbapenemase at low level. Use of an adequate inoculum (1 öse of 10 μ l) is critical to detect those low-level carbapenemase producers. Another biochemical technique is the β -Carba test based on the hydrolysis of chromogenic carbapenem (Bio-Rad, Cressier). Whereas this test is specific and sensitive, it does not detect rare class A carbapenemases such as FRI-1, IMI-1, NmCA and Sme-1. Both techniques do not need any additional equipment for obtaining the results.





Figure 1. Rapidec Carba NP test

• Mass spectrometry detection of carbapenemase activity may be performed.

A kit has been commercialized under the trading name MBT STAR-Carba IVD Kit (Bruker Daltonics). It possesses good specificity and sensibility with acquisition of rapid results but requires some expertise to be implemented by any MALDI-TOF owner.

• The CIM test. The principle of this test is based on putting a meropenem-containing disk in a liquid medium for 2 h at 37° C with the strain to be tested (Fig. 2). Then the disk is removed and further tested by performing a regular disk diffusion antibiogram with a meropenem-susceptible *E. coli* strain as the indicator strain. If the disk still contains a meropenem activity, it will inhibit the growth of the *E. coli* strain indicating that the original tested strain did not produce any carbapenemase activity. If no growth of the indicator strain is visible, the tested strain is a carbapenemase producer. This test is a modified version of the antique Hodge test. It is cost-effective, sensitive and specific. However, it is time consuming (additional 24 h of culture) that does not fit the requirement of rapid detection of carbapenemase activity.



4- Immunological detection of carbapenemases. Lateral flow techniques have been commercialized for detecting the most common carbapenemases of the KPC, NDM, VIM, OXA-48 types. Two companies, namely Coris Bioconcept (Gembloux, Belgium) and NG Biotech (Guipry, France) have marketed recently those lateral flow techniques. The test developed by NG Biotech additionally detects IMP producers. Both tests are rapid (15 min), sensitive and specific. They do not need any additional equipment for their reading.



Figure 3. Lateral-flow techniques for detecting carbapenemases

5- Molecular identification of carbapenemase genes. PCR and sequencing are the cornerstone of identification of carbapenemase genes. Whereas home-made techniques may be used, many commercially available tests are now available such as the XpertCarba-R, (Cepheid) Amplidiag CarbaR+VRE, Amplidiag CarbaR+MCR (Mobidiag) Check Direct CPE on BD MAXTM (Check-Points) eazyplexSuperBug CRE (OptiGene) Carbaplex IVD PCR (Brucker), CRE ELITe MGB Kit (ELITech), BioFire Multiplex Film Array BCDI2 Panel (bioMérieux), Revogene (GenePOC). Those tests possess high sensitivity and specificity. Some of them can be used directly with clinical samples such as rectal swabs, stools or blood cultures. They are design to identify known genes. Their use requires additional equipment, significantly impacting the cost effectiveness. Furthermore, the cost of each test remains significant.

Whole genome sequencing (WGS) may also be used, in particular for detecting unknown carbapenemase genes once a carbapenemase producer is identified using non-molecular techniques, but molecular tests remain negative. WGS so far can only be performed by expert laboratories and remains neither cost effective nor rapid.

We recommend therefore the following strategy for detection of carbapenemase producers;

- 1- Identification and susceptibility testing
- 2- Detection of biochemical activity (Rapid Carba NP; β-Lacta test)
- 3- Immunological detection of the carbapenemase group
- 4- PCR and sequencing for the detection of carbapenemase genes

NB; several techniques are being evaluated for detection of carbapenemase producers directly from infected samples (blood cultures, urine, pulmonary sample....). However, no consensus exists currently for carbapenemase detection directly from those clinical samples.

B - Screening (colonization)

1- Which patients to be screened?

The Swiss noso guidelines may be followed

(https://www.swissnoso.ch/fileadmin/swissnoso/Dokumente/6_Publikationen/Bulletin _Artikel_F/170606_Olearoetal_FR.pdf)

It seems reasonable to screen the following patients;

- Patients hospitalized abroad and transferred directly to an hospitalization unit in Switzerland
- Contact patients of a patient colonized or infected with a CPE
- Patients hospitalized in at risk units such as ICU

2- Which samples ?

- Stools
- Rectal swabs of good quality Repeated screening of samples may be recommended if patients are treated with antibiotics (false negatives samples) and for high risk patients.

3- Screening culture media

- Several screening media have been developed for screening CPE. Not all of them may screen for all types of CPE.

The ChromID[®] Carba SMART (bioMérieux) and mSuperCARBA (CHROMagar) can screen any type of carbapenemase producer. Actually ,the Carba SMART is a biplate that contains on one side a carbapenem molecule and on the other side temocillin, taking in a account that OXA-48 producers are temocillin resistant. Indeed, due to a low-level resistance to carbapenems observed for some OXA-48 producers, their detection on the carbapenem-containing side of this medium may remain negative.

- CHROMagarTM KPC (CHROMagar)
- ChromID[®] Carba (bioMérieux)
- Brilliance[®] CRE (Oxoid)
- ChromID[®] OXA-48 (bioMérieux)
- The ChromID[®] Carba SMART (bioMérieux)
- mSuperCARBATM (CHROMagar)
- ChromID[®] Carba SMART (bioMérieux)
- Following the screening process, detection of carbapenemase activity, immunological and molecular identification of the carbapenemase types will be done as indicated above
- Direct molecular detection of carbapenemase genes has been proposed Xpert[®] Carba-R, (Cepheid)Amplidiag[®] CarbaR+VRE, Amplidiag[®] CarbaR+MCR (Mobidiag) Check Direct CPE on BD MAXTM (Check-Points) eazyplex[®] SuperBug CRE (OptiGene) Carbaplex[®] IVD PCR (Brucker). CRE ELITe MGB[®] Kit (ELITech). Those techniques are generally considered are more sensitive that the culture screening techniques. However false negative and false positive results have been reported. Therefore, concomitant screening by cultures is recommended
- Recommendations
 - Non outbreak situation (Fig 4); direct plating of rectal swabs or stools on chromogenic selective media.
 - Outbreak situation (Fig. 5)
 - Direct plating on chromogenic selective media and/or use of molecular techniques
 - Concomitant cultures of the samples in an enrichment broth containing ertapenem 0.5 mg/L to improve the sensitivity of the detection



Figure 4. Carbapenemase detection of carriage (in the <u>absence</u> of an outbreak)



Figure 5. Carbapenemase detection of carriage (in the presence of an outbreak)

Recommended reading

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