Strategies for identification of carbapenemase-producing Enterobacteriaceae

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The spread of carbapenem-hydrolysing β-lactamases in Enterobacteriaceae is becoming a major public health issue. These β-lactamases hydrolyse almost all β-lactams, are plasmid-encoded and are easily transferable among enterobacterial species. They are found in multidrug-resistant isolates. Detection of these isolates from infected specimens first relies on careful recognition of any decreased susceptibility to carbapenems. After this, rapid biochemical identification of carbapenemase producers using the novel Carba NP test should be performed. Subsequently, molecular techniques can be used to identify carbapenemase genes if necessary (epidemiology). Detection of carriers relies on a preliminary screening step, with stools or rectal swabs being screened on selective culture media such as SUPERCARBA medium, which possesses the broadest spectrum for detecting any type of carbapenemase producer.

Keywords: antibiotics, carbapenems, β-lactams, resistance, Gram-negatives

Introduction

Multidrug resistance is now emerging increasingly in Enterobacteriaceae among nosocomial and community-acquired infections. One of the most important emerging resistance traits corresponds to the production of the carbapenem-hydrolysing β-lactamases, which confer resistance to almost all β-lactams.¹ Their current and extensive worldwide spread in Enterobacteriaceae is an important source of concern since these carbapenemases are, in most cases, combined with non-β-lactam resistance mechanisms, therefore leading to multidrug-resistant isolates.¹ The high rate of transmissibility of the carbapenemase genes, which are mostly located on self-conjugative plasmids carrying other resistance determinants, explains the need to rapidly identify the carbapenemase producers in order to guide antibiotic therapy but also to prevent the development of outbreaks.

Categories of carbapenemases and their properties

Carbapenemases in Enterobacteriaceae are currently mostly of the KPC, VIM, IMP, NDM and OXA-48 types, and their detailed properties have been extensively reported.¹ KPC enzymes hydrolyse all β-lactams (although they hydrolyse cephemycins at a low level) and their activity is only inhibited partially in vitro by clavulanic acid, tazobactam and boronic acid. The metallo-β-lactamases (MBLs; IMP, VIM and NDM) hydrolyse all β-lactams except aztreonam and their activity is not affected by any of the inhibitors that are in clinical use, but they can be inhibited in vitro with compounds such as zinc chelators (EDTA, for example). The OXA-48-type enzymes hydrolyse aminopenicillins, ureidopenicillins and carbapenems at low levels, but do not significantly hydrolyse broad-spectrum cephalosporins.¹² Their activity is not affected by the inhibitors in clinical use, but they are inhibited by NaCl in vitro.²

Susceptibility breakpoints and the debate around detection

Both the US and European (EUCAST) breakpoints for carbapenems have been lowered recently to permit better recognition of carbapenem-resistant isolates.³⁴ The detection of carbapenemase producers in clinical specimens is based first on the analysis of susceptibility testing. According to the US guidelines (CLSI) updated in 2012,¹ the breakpoints for imipenem and meropenem are susceptible (S) ≤1 and resistant (R) ≥4 mg/L, and for ertapenem S ≤0.5 and R ≥2 mg/L. Ertapenem seems to be a good candidate for detecting most of the carbapenemase producers since MIC values of ertapenem are usually higher than those of other carbapenems.² However, detection of carbapenemase producers based only on MIC values may lack sensitivity. According to the CLSI and EUCAST guidelines, breakpoints have to be considered only when reporting susceptibility or resistance to carbapenems.⁵⁶ Special tests for carbapenemase detection are suggested for epidemiology and infection control issues only.⁵⁶
We actually disagree with these guidelines, for the following reasons: (i) susceptibility to carbapenems is observed for several carbapenemase producers; and (ii) there is a paucity of clinical successes of carbapenem-containing regimens for treating infections due to carbapenemase producers that are susceptible to carbapenems in vivo. Based on our own experience, we propose that, as a minimum, detection of carbapenemase activity should be performed on enterobacterial isolates exhibiting MIC values of ertapenem ≥0.5 mg/L or of imipenem or meropenem ≥1 mg/L, and also on any enterobacterial isolate that exhibits even a slight decrease in susceptibility to carbapenems compared with a wild-type phenotype. This detection will be useful for treating patients and for preventing nosocomial outbreaks of carbapenemase producers (and therefore multidrug-resistant isolates), whatever the carbapenem resistance level is.

Non-molecular tests for carbapenemase production

A series of non-molecular tests have been proposed for detection of carbapenemase activity. Some have good specificity and sensitivity, but none of them approaches 100%. The modified Hodge test (MHT), based on in vivo production of a carbapenemase, has been used for years. Addition of zinc to the culture medium has been shown to improve the sensitivity of this test. However, we believe that this technique is time-consuming and shall be discarded. In addition, MHT may lack sensitivity for detecting carbapenemase activity in Enterobacter spp. The added value of the inhibition-based carbapenemase detection tests remains variable. These tests are, for example, based on inhibition by tazobactam, clavulanic acid or boronic acid for detecting the production of Ambler class A carbapenemases (KPC), and inhibition by EDTA or dipicolinic acid for detection of MBL activity. They are time-consuming and have variable sensitivity and specificity. In addition, they require trained microbiologists. High-level resistance to temocillin has been suggested to be predictive of production of OXA-48, but the specificity of this feature remains to be more extensively evaluated.

Detection of carbapenemase activity can be done using a UV spectrophotometer, which is available in many microbiology laboratories. It is based on several steps, including: (i) an 18 h culture (which can be shortened in some cases to 8 h); (ii) a protein extraction step; and (iii) measurement of imipenem hydrolysis using a UV spectrophotometer. We have shown that this spectrophotometry-based technique has 100% sensitivity and 98.5% specificity for detecting any kind of carbapenemase activity. This cheap technique can accurately differentiate carbapenemase producers from non-carbapenemase producers among carbapenem-non-susceptible isolates (outer membrane permeability defect, overproduction of cephalosporinases or extended-spectrum β-lactamases (ESBLs)). It can be implemented in any reference laboratory, but this technique still requires time. Recently, the use of mass spectrometry for detection of carbapenemase activity has been proposed, based on the analysis of the degradation of a carbapenem molecule. Although this technique has to be further evaluated, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) equipment is increasingly used in the diagnostic bacteriology laboratory.

The most important and recent development for the accurate identification of carbapenemase-producing Enterobacteriaceae is the Carba NP test. This biochemical test, applicable to isolated bacterial colonies, is based on in vitro hydrolysis of the carbapenem imipenem. Hydrolysis of imipenem is detected by a change in the pH value of the indicator (red to yellow/orange). This test is 100% sensitive and specific, as are molecular techniques. It detects not only all known carbapenemases (belonging to Ambler A, B and D classes) in Enterobacteriaceae but should also identify virtually any new emerging carbapenemase, in contrast to molecular techniques. This rapid (<2 h), user-friendly and inexpensive technique can be implemented in any laboratory worldwide. In addition, it does not require any specific equipment. We believe this technique will soon become a reference technique since it fulfils the clinical requirement of a rapid and low-cost identification method for carbapenemase-producing Enterobacteriaceae.

Molecular tests for carbapenemase genes

Molecular techniques remain the gold standard for the precise identification of carbapenemase genes. Most of these techniques are based on PCR and may be followed by a sequencing step if a precise identification of the carbapenemase gene is needed (e.g. VIM type, KPC type, NDM type or OXA-48 type). They are either single or multiplex PCR techniques. A PCR technique performed directly on colonies can give results within 4–6 h (or less when using real-time PCR technology) with excellent sensitivity and specificity. Similarly, other molecular techniques are useful for this purpose. The main disadvantages of the molecular-based technologies are their cost, the requirement for trained microbiologists and the inability to detect novel unidentified genes. Sequencing of the genes is interesting mostly for research and epidemiological purposes. Precise identification of the type of carbapenemase is not actually needed for treating patients or for preventing outbreaks. We believe these molecular techniques may be mostly used in reference laboratories.

Screening of colonized patients

Preventing the spread of carbapenemase producers relies on the accurate detection of colonized patients at an early stage of hospitalization or on admission/discharge either to the hospital or to a specific unit. Screening should include as a minimum ‘at-risk’ patients, such as those in intensive care units, transplant recipients and the immunocompromised, and those transferred from any foreign hospital (unknown prevalence of carbapenemase producer carriage) or from non-foreign hospitals but known to face a high risk of carriage of carbapenemase producers. Geographical regions with high prevalence of carbapenemase producers are increasingly known. Since the reservoir of Enterobacteriaceae is mostly the intestinal flora, stools and rectal swabs are the most suitable specimens for performing such screening. Three screening media are currently known, but mostly for carbapenem-resistant rather than carbapenemase-producing organisms.

The first marketed screening medium was the CHROMagar KPC medium, which contains a carbapenem (CHROMagar, Paris, France). It detects carbapenem-resistant bacteria only if they exhibit high-level resistance to carbapenems. Its main disadvantage therefore remains its lack of sensitivity since it does not detect carbapenemase producers exhibiting a low level of...
carbapenem resistance, as observed for several MBL or OXA-48 pro-
ducers. The second screening medium also contains a carbapenem
(CRE Brilliance, Thermo Fisher Scientific, UK).14 It detects KPC and MBL
producers well, and most but not all OXA-48 producers.16,15 Finally,
one of the most recently developed screening media (SUPERCARBA)
contains claxacillin, zinc and ertapenem.15 It shows excellent sensiti-
ity and specificity for detection of any kind of carbapenem produ-
cer (not only high-level carbapenem-resistant isolates).16 Compared
with the two other media, it also shows improved sensitivity and spe-
cificity for detecting all types of carbapenemase producers (including
the OXA-48 producers) when present in low amounts in stools. Once
carbapenem-resistant isolates are selected on SUPERCARBA
medium, we recommend use of the Carba NP test for detecting
carbapenemase activity. Then, if needed, molecular identification of
the carbapenemase genes may be performed.

Conclusion

Proposed algorithm for carbapenemase detection

We propose the following scheme for detection of carbapenem-
ase producers in Enterobacteriaceae.

(i) Infecting strains: Carba NP test on isolated colonies with
decreased susceptibility to carbapenems. If positive, molecular
identification of the genes, mainly for epidemiological reasons.

(ii) Screening of carriers: screening of carbapenem-resistant
isolates using e.g. SUPERCARBA medium, followed by the Carba
NP test to be performed on selected colonies. If the latter test is
positive, molecular identification of the genes, mainly for
epidemiological reasons.

The proposed scheme for detection of carbapenemase produ-
cers presents several advantages. It will lead to rapid identifica-
tion of carbapenemase producers, allowing adequate antibiotic
stewardship. This is of the utmost importance due to the current
shortage of novel antibiotics. It will prevent the develop-
ment of nosocomial outbreaks due to the multidrug-resistant
bacteria that represent one of the most worrisome medical
threats. The Israeli experience with containment of the KPC
outbreaks has demonstrated the possibility of obtaining frank
success by acting rapidly, and on a large scale.17 This strategy,
based on broad and rapid detection of carbapenemase producers,
can also have a significant impact in preventing their
spread in the community. Finally, the first proposed steps
(screening, susceptibility testing and Carba NP test), excluding
the molecular techniques (needed for epidemiological purposes),
can be implemented immediately in any laboratory worldwide,
since they are based on inexpensive and affordable techniques.

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References

1 Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing
2 Nordmann P, Gniadkowski M, Giske CG et al. Identification and screening of
carbapenemase-producing Enterobacteriaceae. Clin Microbiol Infect 2012; 18:
432–8.
3 Clinical and Laboratory Standards Institute. Performance Standards for
Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement
4 Leclercq R, Canton R, Brown DF et al. EUCAST expert rules in antimicrobial
5 Girlich D, Poirel L, Nordmann P. Value of the modified Hodge test for
detection of emerging carbapenemase-producing Enterobacteriaceae. J Clin
6 Berneau S, Poirel L, Nordmann P. Spectrophotometry-based detection of
carbapenemase producers in Enterobacteriaceae. Diagn Microbiol Infect Dis
2012; 74: 88–90.
7 Burckhardt I, Zimmermann S. Using matrix-assisted laser desorption
ionization-time of flight mass spectrometry to detect carbapenem
8 Hrabak J, Walkova R, Studentova V et al. Carbapenemase activity
detection by matrix-assisted laser desorption ionization-time of flight
9 Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-
10 Poirel L, Walsh TR, Cuvillier V et al. Multiplex PCR for detection of acquired
11 Avlamis A, Bekris S, Ganteris G et al. Detection of metallo-
β-lactamases genes in clinical specimens by a commercial multiplex PCR
12 Cuzon G, Naas T, Bogaerts P et al. Evaluation of a DNA microarray
for the rapid detection of extended-spectrum β-lactamases (TEM, SHV
and CTX-M), plasmid-mediated cephalosporinases (CMY-2-like, DHA, FOX,
ACC-1, ACT/MIR and CMY-1-like/MOX) and carbapenemases (KPC,
13 Moran-Gilad J, Carmeli Y, Schwartz D et al. Laboratory evaluation of
the CHROMagar KPC medium for identification of carbapenem-non
14 Withey S, Scopes E. A new screening medium for detection of
 carbapenem-resistant Enterobacteriaceae. In: Abstracts of the
Twenty-first European Congress for Clinical Microbiology and Infectious
Diseases, Milan, 2011. Abstract P662. European Society for Clinical
Microbiology and Infectious Diseases, Basel, Switzerland.
15 Girlich D, Poirel L, Nordmann P. Strategy of detection of
 carbapenemase-producing Enterobacteriaceae. In: Abstracts of the
Twenty-second European Congress for Clinical Microbiology and Infectious
Microbiology and Infectious Diseases, Basel, Switzerland.
16 Nordmann P, Girlich D, Poirel L. Detection of carbapenemase
producers in Enterobacteriaceae by use of a novel screening medium.
outbreaks of carbapenem-resistant Klebsiella pneumoniae in Israeli hospitals