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Review

How to detect carbapenemase producers? A literature review of phenotypic and molecular methods

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ABSTRACT

The production of carbapenemases by Gram negative bacterial pathogens has become a worldwide threat to successful antibiotic therapy. Carbapenem resistance has been increasingly reported in recent years, and given the paucity of reliable antimicrobials, focus has shifted towards early surveillance of carbapenemases in microbiology laboratories. Detection of carbapenemases is primarily based upon careful recognition of decreased in vitro susceptibility to carbapenems by measurement of their MIC values or inhibition zone diameters. This is followed by a set of conventional phenotypic methods of variable efficiencies, such as the modified Hodge test and culture-based tests utilizing carbapenemase inhibitors. Among these, boronic acid compounds are used to inhibit Ambler class A carbapenemases, and EDTA and dipicolinic acid are used to inhibit Ambler class B carbapenemases. While the detection of carbapenemase producers is possible using screening culture media, the identification of carbapenemase genes relies on molecular techniques. Polymerase chain reaction experiments allow the detection of well-known carbapenemase genes, and sequencing is essential to the identification of new genes. Innovative biochemical and spectrometric detection are being developed to complement the molecular methods and shorten processing times needed for detection of carbapenemase activity. These are promising options to become routinely applied for rapid detection of carbapenemase-producing organisms with high precision and are most useful for epidemiologic purposes. Molecular techniques are nevertheless expensive, time consuming, and require well-trained personnel. This review is a summary of the current state-of-art of carbapenemase detection methods, with a description of the advantages and limitations of each.

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1. Introduction

Carbapenemases are emerging resistance determinants in Gram negative pathogens, including *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. These carbapenem-hydrolyzing enzymes confer resistance to a broad variety of β-lactams, and are located on self-conjugative plasmids carrying other resistance determinants, capable of disseminating among bacteria and resulting in spread of resistance to multiple classes of antibiotics like fluoroquinolones, aminoglycosides and cotrimoxazole (Falagas et al., 2011; Nordmann and Poirel, 2013).

The first carbapenemase was described in France in the year 1993 and its gene bla_{NmcA} was localized on the chromosome of an *Enterobacter cloacae* (Naas and Nordmann, 1994). In 1995, a report from Japan described a plasmid-borne gene bla_{IMP-1} capable of hydrolyzing carbapenems in *Serratia* (Ito et al., 1995). In 2001, a similar report from North Carolina documented another plasmid-borne, carbapenem-hydrolyzing gene bla_{KPC-1} that was recovered from an isolate of *Klebsiella pneumoniae* (Yigit et al., 2001). Since then, a number of newly recognized carbapenemases has proliferated and disseminated generating a major therapeutic and epidemiological concern, due to restriction in patient treatment options and infection control strategies (El-Herte et al., 2012). Carbapenems are indeed broad spectrum antibiotics that represent key agents in life-threatening nosocomial infections, transplantations, hospitalizations in intensive care units, and surgeries (Nordmann et al., 2012a). Their use has increased in clinical practice as a result of expanding resistance to other β-lactam antibiotics, being the sole antibiotics of this class with proven efficacy against extended-spectrum β-lactamase (ESBL) producing Gram negative bacteria (Hawkey and Livermore, 2012). Resistance to carbapenems has been largely reported as a consequence of acquisition of carbapenemase-encoding genes, even though other resistance mechanisms, such as reduced permeability of the outer membrane due to porin alterations, or high efflux pump activity, may be responsible of carbapenem resistance (Nordmann et al., 2011a).

The clinically significant carbapenemases belong to Ambler class A (KPC, GES) with serine active sites and low hydrolysis of all β-lactams except cephamycins; or Ambler class B (NDM, VIM, and IMP) which are zinc-dependent metallo-β-lactamases (MBLs) strongly hydrolyzing all β-lactams except aztreonam; or Ambler class D (OXA-type) with weak hydrolysis of carbapenems, and no effect on broad spectrum cephalosporins and aztreonam (Queenan and Bush, 2007; Poirel et al., 2012). The Ambler class C cephalosporinases (AmpC cephalosporinases) have very little hydrolytic activity, if any, against carbapenems (Jacoby, 2009). However, the production of plasmid-encoded AmpC cephalosporinases has emerged as an important resistance determinant to carbapenems in isolates with impermeability mechanisms or efflux pump overactivity (Gutierrez et al., 2007; Dahmen et al., 2012). A comparison of the clinically significant carbapenemases is shown in Table 1.

Because carbapenemases represent a versatile family of β-lactamases of increasing incidence, the optimization of their detection techniques is necessary, an action that may be challenging for the microbiology laboratory (Queenan and Bush, 2007). The effective and timely detection of carbapenemase-producing organisms is an urgent issue, not only for the selection of appropriate therapeutic schemes but also for the implementation of infection control measures. However, this detection includes a number of difficulties, because it cannot be simply based on resistance profile, and its accurate methodology has not been yet adequately standardized (Miriagou et al., 2010). In general, a preliminary screening of carbapenemase producers relies on recognition of decreased susceptibility to carbapenems in antibiotic susceptibility tests, followed by other phenotypic and biochemical tests (Cohen Stuart and Leverstein-Van Hall, 2010). However, and although not available in many laboratories, carbapenemase gene recognition by molecular methods remains the gold standard of detection (Nordmann and Poirel, 2013). Other newer, alternative techniques based on analytical methods or innovative technologies are also being developed and show potential for use due to high efficiency. The purpose of this review is to summarize available methods that have been proposed for laboratory identification of carbapenemase-producing Gram negative bacteria. A scheme for possible utilization of the different methods is presented in Fig. 1. The article constitutes a relevant laboratory tool for the detection of carbapenemases and opens wide perspectives in clinical and experimental microbiology.

2. Screening of carbapenemase-producers based on antibiotic susceptibility tests

The first cause of suspicion of carbapenemase production in a clinical isolate is an increase in carbapenem minimum inhibitory concentration (MIC) or a decreased in inhibition zone diameter. This result renders a bacterial isolate eligible for further analysis for carbapenemase production using more specific methods (Miriagou et al., 2010). The carbapenem susceptibility ranges for *Enterobacteriaceae*, *Pseudomonas*, and *Acinetobacter* are shown in Table 2.

According to the 2014 recommendations of the European Community on Antimicrobial Susceptibility testing (EUCAST), the MIC breakpoints of imipenem and meropenem for *Enterobacteriaceae* are greater than 8 mg/L, while the MIC breakpoint of ertapenem is greater than 1 mg/L (EUCAST, 2014). According to the 2014 US guidelines of the Clinical Laboratory and Standards Institute (CLSI), these breakpoints are greater than or equal to 4 mg/L for imipenem and meropenem and greater than or equal to 2 mg/L for ertapenem (CLSI, 2014). Ertapenem seems to be a good candidate for detecting most carbapenemase producers among *Enterobacteriaceae* because MIC values of ertapenem are usually higher than MICs of other carbapenems (Nordmann et al., 2012c). However, detection of carbapenemase producers based only on MIC values may lack sensitivity. Many carbapenemase producing *Enterobacteriaceae* show broad range of MICs with sometimes values within the susceptibility range. Indeed, intermediate susceptibility, or even sensitivity to carbapenems has been observed for producers of all types of carbapenemases especially the OXA-48/OXA-181 producing *Enterobacteriaceae* that do not co-harbor an ESBL (Table 3) (Nordmann, 2010; Nordmann et al., 2011a). Carbapenem MICs are expected to substantially rise only in the presence of an additional resistance mechanism, like permeability lesions due to outer membrane protein mutation, or simultaneous production of AmpC cephalosporinases or ESBLs (Livermore and Woodford, 2006). To avoid false negative results, or to maximize detection sensitivity, it has been proposed to screen enterobacterial isolates for carbapenemase activity if they exhibit MICs of ertapenem greater than or equal to 0.5 mg/L or MICs of imipenem or meropenem greater than or equal to 1 mg/L, or to screen any enterobacterial isolate displaying a slight decrease in susceptibility to

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