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Detection of different classes of carbapenemases: Adaptation and assessment of a phenotypic method applied to *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, and proposal of a new algorithm

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ABSTRACT

A new phenotypic method for detecting carbapenemases has been adapted (assembling of two MAST® kits, including one that contains faropenem to which a temocillin disk has been added) then assessed using 101 bacterial strains (*Enterobacteriaceae* with assays on *Pseudomonas aeruginosa* and *Acinetobacter baumannii)* including 62 which produce genetically identified carbapenemases. Concerning Carbapenemase-Producing *Enterobacteriaceae* (CPE), there is 100% sensitivity for *Klebsiella pneumoniae* carbapenemase (KPC, Ambler class A) and OXA-48 (Ambler class D), and 91% for metallo-beta-lactamase (MBL, Ambler class B), with a 97% sensitivity for all carbapenemases, with a specificity of 100%. The test is also efficient for detecting *Pseudomonas aeruginosa* carbapenemases (sensitivity between 82 and 100% and 100% specificity). The major innovation is the combined use of faropenem and temocillin for reliable detection (excellent performance with 100% sensitivity and specificity) of OXA-48. This study has led to the development of a new algorithm to detect the different classes of carbapenemases, for first-line diagnosis, by combining this modified MAST® test with immunochromatographic methods and molecular biology techniques.

1. Introduction

Clinical microbiologists are frequently confronted with problems relating to hospital hygiene and in particular threats of epidemics at all levels: detection, diagnosis, epidemiological investigations, management, reporting, infection control and prevention, antibiotic stewardship and therapeutic advice for patient care.

Among the many risks of bacteriological and nosocomial outbreaks and infections (Logan and Weinstein, 2017), Carbapenemase-Producing *Enterobacteriaceae* (CPE) are currently one of the major concerns relative to infectious diseases (Nordmann et al., 2011). In fact, this enzyme production, which also concerns *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, creates situations of antibiotic resistance leading to loss of therapeutic options that currently represent one of the most alarming dangers affecting worldwide public health. The reasons for this emergence and the difficulties of controlling spread of carbapenemases are multiple and multifactorial, and must be viewed in parallel with the slowdown of research and rollout of new antibiotics (Elhani, 2011).

Faced with an increase in morbidity, mortality and the cost of additional care relative to this global spread (Nordmann et al., 2011; Magiorakos et al., 2013; Djahmi et al., 2014; Hrabak et al., 2014;

Abbreviations: cAmpC, chromosomal hyperproduced cephalosporinase(s); CAT, carbapenemase activity test; Col iz, colony (colonies) within inhibition zone; FP, false positive(s); hAmpC, hyperproduced cephalosporinase(s); IC, immunochromatography; MBL, metallo-beta-lactamase(s); pAmpC, plasmid-mediated hyperproduced cephalosporinase(s); TEM, temocillin; TN, true negative(s)

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Freeman et al., 2016), the current priority, in order to address the deficiencies of the therapeutic arsenal available today, is to prevent dissemination of these resistance enzymes. The first essential step is early, simple and reliable detection. The phenotypic and/or genotypic methods must be standardized and accessible to all microbiology laboratories. Therefore, it is a capital issue for worldwide public health to detect carbapenemases to issue alerts and ensure immediate implementation of strict and efficient hospital hygiene measures, in order to prevent and contain outbreaks and to stop the spread of these resistance enzymes. They are polymorphous enzymes subdivided into three classes: A, B and D, according to the Ambler classification (Nordmann and Carrër, 2010). Phenotypic detection of OXA-48 and OXA-48-like, enzymes prevalent in many countries of the world (Nordmann et al., 2011; Djahmi et al., 2014), particularly in Europe and the Mediterranean area (Poirel et al., 2004; Benouda et al., 2010; Carrër et al., 2010; Cuzon et al., 2011; Moquet et al., 2011; Nordmann et al., 2011; Poirel et al., 2011c; Vaux et al., 2011; Boutet-Dubois et al., 2012; Glasner et al., 2013; Djahmi et al., 2014) and including France (Poirel et al., 2011a; Mairi et al., 2017), is often complicated and virtually impossible due to the absence of a specific inhibitor.

The object of this study is to adapt and assess a new phenotypic method for detecting the three classes of carbapenemases, then to associate it with other available methods in a new algorithm for the purpose of quick diagnosis. This new test is an assembly of five disks from two different MAST® kits (combined-discs tests and a disk of faropenem; Mast-Diagnostic, Amiens, France) with an additional disk of temocillin, meaning six disks placed at equal distances.

The intended objectives of this approach is to ensure by means of a simple phenotypic test combining several disks:

- the correct detection of carbapenemases (with satisfactory sensitivity and specificity),
- the determination of the detected class of carbapenemase (A (KPC), B or D (OXA-48)),
- the detection of class D carbapenemases, in particular OXA-48 (usually impossible to detect with currently available kits of combined disks; previous studies have reported the efficiency of MALDI-TOF mass spectrometry meropenem hydrolysis assays (Oviano et al., 2016)),
- the possibility of applying this test to strictly aerobic Gram-negative bacilli (*P. aeruginosa* and *A. baumannii*),
- the detection of certain types of carbapenemases within a specific class (class A enzymes other than KPC and class D other than OXA-48),
- the assessment of the ability of this phenotypic test to identify hyperproduced cephalosporinases (hAmpC) in addition to distinguishing them from carbapenemases.

Choosing to combine faropenem and temocillin meets this objective of reliable detection of class D carbapenemases, and OXA-48 in particular. Faced with the inability of detecting OXA-48 using currently available combined disk kits, and the difficulties encountered using the many other phenotypic tests (Miriagou et al., 2010; Poirel et al., 2010b; Nordmann et al., 2011; Birgy et al., 2012; Boutet-Dubois et al., 2012; Nordmann et al., 2012a; Poirel et al., 2012; Dortet et al., 2014; Hammoudi et al., 2014; Hrabak et al., 2014; Bakthavatchalam et al., 2016; Mairi et al., 2017), the main objective of this study remains the accurate detection of OXA-48 using a combined disk method and assess its sensitivity and specificity.

2. Material and methods

2.1. Selection of bacterial strains and resistance mechanisms panel

One hundred and one genetically identified non-duplicate bacterial strains were tested (83 *Enterobacteriaceae*, 12 *P. aeruginosa* and six *A. baumannii* identified using MALDI-TOF mass spectrometry (Bruker Daltonics (Billerica, United States of America) mass spectrometer and database)), in order to determine the ability to detect carbapenemases (and to a lesser extent hAmpC) using this method, by comparing the strains of interest with various control strains.

Sixty two bacterial strains (48 *Enterobacteriaceae*, eight *P. aeruginosa* and six *A. baumannii*) producing carbapenemases (six class A (five KPC-1, one GES-11), 25 class B (six VIM-1, three VIM-2, two IMP-1, 14 NDM-1), 31 class D (26 OXA-48 including 10 associated to ESBL (Extended Spectrum Beta-Lactamases, CTX-M-1), two OXA-23, two OXA-24, one OXA-58)) were tested and compared to 39 other strains (including 28 resistant to carbapenemases) among which were two cases of Pandrug-Resistant (PDR) bacteria and 46 having the following resistance mechanisms: 14 hAmpC (11 chromosomal (cAmpC) and three plasmid-mediated (pAmpC) including one DHA-1), one OXA-48-like without carbapenemase activity (OXA-405), two non carbapenemase oxacillinases (one OXA-4, one OXA-9), three cephalosporinases (one CMY-2, two DHA-1), 11 changes in permeability (mutation of porins OmpC, OmpF or of efflux pump AcrB), two penicillinases (one TEM-1, one SHV-1) and 13 ESBL (12 CTX-M-1, one PER-1).

2.2. Beta-lactamase identification

After susceptibility testing using the diffusion method on Mueller-Hinton (MH) agar plates (Biomérieux, Marcy l'Etoile, France), with or without cloxacillin 250 mg/L, identification was obtained through multiplex PCR then sequencing and comparison with the database (National Center for Biotechnology Information (NCBI), GenBank®) to provide reference for this test. The distribution of bacterial and enzymatic panels will be detailed further.

2.3. Adapting and performing the phenotypic test

The phenotypic test evaluated here was designed by assembling two kits from MAST (Mast-Diagnostic, Amiens, France) then adapted by adding a temocillin disk (30 µg) (Fig. 1). Therefore it was based on a kit composed of combined disks (MASTDISCS® Combi Carbapenemase Detection Set (D70C) (Mast Diagnostic, 2013) containing a carbapenem (disk A, 10 µg meropenem only) with three of the disks containing meropenem associated with various inhibitors (disk C for KPC, disk B for MBL, disk D for hAmpC). One kit was adapted to include a disk impregnated with faropenem (MASTDISCS® ID Carbapenemase Activity Test (CAT) discs (D71C) (Mast Diagnostic, 2014)) with an additional temocillin disk, on a MH agar plate (pure culture, 0.5 MacFarland, according to the manufacturer's recommendations), followed by aerobic incubation at 37 °C for 18 to 24 h, then interpretive reading by a single operator (absence of inter-individual variation).

Test reading is based on measuring the differences in diameter between disks A, B, C and D (Table 1, Fig. 2).

2.4. Determining sensitivity and specificity

Sensitivity and specificity were assessed for each class of enzyme and were also detailed for each bacterial strain. Performance values were thus determined for all carbapenemases.

Concerning results with high phenotypic orientations (extremely close to the set criteria), sensitivity values were provided on an indicative basis by rejecting and accepting them (extreme values in brackets). The selected sensitivity value is the median one.

3. Results

The overall sensitivity of detection of all carbapenemase classes (A, B and D, pre-selected collection) for this test on *Enterobacteriaceae* was 97% [96–98%]. The specificity was 100% (panel of 96 enzymes: 48 carbapenemases, 14 hAmpC, 28 others and six negative strains)