LETTER TO THE EDITOR

Thierry NAAS[1,2,3,4*]

[1]*Research Unit EA7361 "Structure, dynamics, function and expression of broad spectrum ß-lactamases", Faculty of Medicine, Université Paris-Sud, Université Paris-Saclay, Le Kremlin-Bicêtre, France*

[2]*Department of Bacteriology-Hygiene, Bicêtre Hospital, Assistance Publique – Hôpitaux de Paris, Le Kremlin-Bicêtre, France*

[3]*Associated French National Reference Center for Antibiotic Resistance, Le Kremlin-Bicêtre, France*

[4]*Joint Research Unit EERA «Evolution and Ecology of Resistance to Antibiotics», Institut Pasteur – APHP - Université Paris-Sud, Paris, France*

We read with great interest the article by Buzilă *et al*. published in the first issue of *Romanian Archives of Microbiology and Immunology* of 2017 [1], providing a review of the most important beta-lactam resistance mechanisms in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* clinical isolates. While we acknowledge the importance of non-fermenters in clinical practice, we wish to highlight the heterogeneity of beta-lactamases (both narrow-, extended-spectrum and carbapenemases) found in these species. The dissemination of carbapenemases among Enterobacteriaceae and non-fermenters is a great clinical concern. The genetic exchanges at the origin of this dissemination need to be addressed and prevented.

At the French National Reference Center (NRC) for Carbapenem Resistant Enterobactericeae (CRE) based at the Centre Hospitalier Universitaire (CHU) - Bicêtre, France, we expertise per year around 3500 Enterobactericeae with reduced susceptibility to at least one carbapenem, which may be the result of either the association of decreased outer-membrane permeability with plasmid-encoded or chromosome-encoded cephalosporinases and/or extended-spectrum beta-lactamase (ESBL), or the production of carbapenem-hydrolyzing enzymes named carbapenemases. In Enterobacteriaceae, the rise of resistance to carbapenems is mainly due to the dissemination of carbapenemase-producers, while bacteria with impaired outer-membrane permeability are less prone to disseminate. Ertapenem is the most sensitive carbapenem for detection of carbapenemases, and thus should always be included when monitoring carbapenem resistance and carbapenemase production. Unfortunately, classic antibiotic susceptibility testing is not totally efficient to detect carbapenemase producing Enterobacteriaceae (CPE). Even with low screening cut-offs defined by the EUCAST (MIC 0.125 mg/L for meropenem or ertapenem and 1 mg/mL for imipenem) nearly 20% of the CPEs are missed, especially OXA-48 producers. In addition, two algorithms based on the recording inhibition diameter around two or three antibiotics have been proposed to directly identify non-CPE among carbapenem resistant Enterobacteriaceae [2]. However, 42% to 66% of Enterobacteriaceae with a reduced susceptibility at least to one carbapenem required confirmatory testing.

In practice, Enterobactericeae with reduced susceptibility to any carbapenem should undergo further confirmatory testing for the presence of a carbapenemase. Thus, simple and reliable confirmatory tests to rapidly discriminate CPE from non CPEs are mandatory. Various phenotypic tests for detecting carbapenemases have been developed, including carbapenemase activity inhibition tests, the modified Hodge test, and the Carbapenem Inactivation Method [3-5]. These tests, even through cheap require at least 16 hours of additional incubation time and most often lack of sensitivity and specificity, (especially for inhibition tests and modified Hodge test). Recently, the rapid carbapenem inactivation method (rCIM) was designed to ameliorate the detection time of the CIM test, through the use of an *Escherichia coli* indicator

**Corresponding author:* Dr. Thierry Naas, Address : Service de Bactériologie-Hygiène, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre, France. Email: thierry.naas@aphp.fr, Tel: +33 1 45 21 29 86, Fax: +33 1 45 21 63 40

strain in liquid media [6]. Owing to the low cost, rapid nature of the test and the lack of the need of any specific equipment, this test could be easily implemented in small laboratories, with low throughput.

Immunochromatographic assays (ICAs), capable of detecting in less than 15 minutes "The Big 5" carbapenemases (OXA-48-like, IMP-like, NDM-like, KPC-like and VIM-like)" have been developed and have shown their usefulness at the NRC. These tests are well adapted to the French epidemiology of CPE since they were able to detect 99.6% of the circulating CPEs in France. Only minor carbapenemases were missed such as IMI, GES-5, SME, FRI, enzymes. These latter could be detected using carbapenem-hydrolyzing activity tests, such biochemical tests (e.g. Carba NP Test and derivatives), and MALDI-TOF mass spectrometry (MALDI-TOF MS) techniques [7]. Thus, combining ICAs with the home-made Carba NP test, a rapid colorimetric test which permits the detection of carbapenem hydrolysis by monitoring acidification of the media, is the strategy used at the F-NRC. Molecular methods are still considered the gold standard of the detection of carbapenemase producers. These tests have the same drawbacks as ICAs (detection only of the Big 5 carbas, and are costly), but are more sensitive and thus directly usable on clinical specimens such as rectal swabs.

At the NRC, an in-house polymerase chain reaction (PCR) is performed, targeting the blaOXA-48-like, blaKPC-like, blaNDM-like, blaIMP-like, blaIMI-like, blaVIM-like and blaGES-like carbapenemase genes. PCR products are then sequenced for characterisation and surveillance. Carbapenemase activity positive isolates with negative PCR results undergo whole genome sequencing and in depth genomic analysis.

As we have previously reported, Romania has high level of carbapenem resistance with an epidemic dissemination of several carbapenemases [8]. Tackling this major public issue will require thorough epidemiological studies on carbapenemase producing Gram negative bacilli along with identification of CPE carriers and of infected patients in order to reinforce hygiene measures. We look forward to further collaborate with and to assist Romanian microbiology laboratories to implement the proper diagnostic tool, best suited for their setting.

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