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Optimization of the rapid carbapenem inactivation method for use with AmpC hyperproducers

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39 Sir,

40 We read with great attention the comments made by Nordmann and Poirel. Our
41 work¹ was meant to challenge the rCIM test with isolates that pose problems for CPE
42 confirmation using many currently available tests, including the Carba NP test, which is
43 considered as the reference method.

44 The rCIM was initially validated in a prospective study on isolates sent to the
45 French National Reference Center (F-NRC) for CPEs, and thus representing the French
46 CPE epidemiology.² Several isolates/resistance mechanisms were identified as being
47 challenging in terms of CPE identification using the rCIM or the Home-made Carba NP,
48 which is the technique of choice of the F-NRC. In order to improve the rCIM, we
49 concentrated on difficult to categorize enzymes from the F-NRC, including Group 3
50 Enterobacterales isolates, which previously tested positive using the Carba NP test, and
51 also rarer enzymes. *Enterobacter kobei* ST125 isolates expressing ACT-28 have
52 previously been shown to give false-positive Carba NP and rCIM results.^{2,3} As ACT-28
53 has very weak carbapenemase activity, is chromosomally-encoded, linked to a specific
54 lineage of *E. kobei* ST125, and that the carbapenem and cephalosporin susceptibility is

55 fully restored on cloxacillin-containing media, it should be “functionally” classified as a
56 chromosomal cephalosporinase with limited carbapenemase activity.^{1,3} To consider these
57 isolates as “true” carbapenemases may lead to isolation of the patient and unnecessary
58 high costs. Thus, including 10 different ACT-28-producers from the F-NRC allowed to
59 confirm the false-positivity rate using the Carba NP and the rCIM tests, and validate the
60 improvement of the rCIM-A, as no false positive results were observed anymore.

61 It was never claimed in the study by Muntean *et al.*¹ that the isolates represented
62 the French CPE epidemiology, but it was clearly stated that “the strength of the study was
63 that broad range of CREs, which included true CPEs as well as AmpC hyperproducers
64 (both natural and acquired) were investigated”. Our work constitutes a “stress-test” of the
65 optimization step of the rCIM, rather than a determination of the sensitivity and
66 specificity of the Carba NP. Moreover, there was no cherry-picking of isolates that would
67 advantage the rCIM, or the rCIM-A over the Carba NP, and the reported sensitivity and
68 specificity of rCIM-A was lower than that originally reported for rCIM, simply due to the
69 challenging isolates tested. All the isolate/enzyme combinations are published, thus
70 allowing the reader to judge if their particular epidemiological situation is amenable for
71 the use of the tests. Of note, we concluded that rCIM is still lacking sensitivity with IMI-
72 and OXA-23-type carbapenemases, which is likely due to their weak hydrolysis of
73 meropenem, unlike imipenem, which is strongly hydrolyzed. This can be of particular
74 importance, regarding certain epidemiological situations.

75 Several slow-hydrolyzing OXA-48 variants (OXA-162, -181, -204, -232, -244)
76 included, as they have been reported to be missed or inconclusively detected by Carba
77 NP,⁴⁻⁶ yet they were correctly identified by rCIM and rCIM-A.^{1,2} While some of these
78 variants are still rare, others such as OXA-244-producers are increasingly encountered.^{6,7}
79 As their under-detection may lead to silent spread, these isolates should be particularly
80 targeted in the process of the validation of a novel CPE confirmation tests.⁶ Even though
81 a sensitivity and specificity of 100% for CPE detection have been initially reported for

82 the Carba NP by Nordmann *et al.*,⁸ other studies have subsequently reported sensitivities
83 (ranging from <70 - 90%), in particular with OXA-48-like-producers, as these enzymes
84 have weak carbapenemase activity as compared to the other carbapenemases.⁴⁻⁶ In
85 addition, it was noticed in the comments that high rates of OXA-23-producing
86 Enterobacterales were included in our study, while in Switzerland, they represent only
87 0.02% of total CPEs. OXA-23 producing *Proteus mirabilis* are increasingly reported and
88 more prevalent than expected.⁹ Indeed the prevalence of OXA-23 recorded at the F-NRC
89 (0.5% of all CPEs), is likely underestimated as a recent French Nationwide study reported
90 26% of OXA-23-producers among Co-Amoxiclav resistant *P. mirabilis*. Difficulties in
91 their detection as a consequence of low carbapenem MICs contributes to their silent
92 spread.⁹

93 Regarding the time to test positivity, we would like to stress out that even though
94 the Carba NP is faster and may provide a positive test result in 15-30 minutes, it still
95 requires up to 2 hours of incubation for some isolates and to confirm a negative test result.
96 The rCIM(-A) may already give a positive result at 2 hours (30 minutes incubation and
97 1.5 hours growth monitoring of the *E. coli* ATCC 25922 indicator strain) using standard
98 routine equipment and reagents (carbapenem disks, Trypticase Soy Broth, a table-top
99 centrifuge, a vortex and a nephelometer). We agree that rCIM requires extra hands-on
100 time, thus making it less attractive for high-throughput laboratories, but it may be
101 appropriate for low throughput and low resource settings^{1,2}. Finally, both assays have
102 similar turn-around-times, as both require overnight bacterial cultures, and the longer
103 detection time of the rCIM is only a small fraction of total reporting time. Moreover, for
104 initiating appropriate antibiotic treatments in critically-ill patients, rapid susceptibility
105 testing using techniques that have been developed and endorsed by EUCAST are crucial,
106 even in low-and middle-income countries.¹⁰

107

108 **Transparency declarations**

109 None to declare.

110

111

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