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Susceptibility testing methods

Evaluation of the performances of Carba NP test and carbapenem inactivation method for the investigation of carbapenemase activity in Escherichia coli and Klebsiella spp. isolates

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Background: The rapid increase in the prevalence of carbapenemase-producing *Enterobacteriaceae* necessitated fast, reliable and cost-effective methods for the detection this resistance mechanism. A number of phenotypic methods were developed, among them Carba NP test is widely used and is recommended by the guidelines of both Clinical and Laboratory Standards (CLSI) and European Committee on Susceptibility Testing (EUCAST). A recently developed phenotypic test called the Carbapenem Inactivation Method (CIM) was presented as a cost-effective and robust screening method to detect carbapenemase activity. In this study, we aimed to evaluate the performances of Carba NP test and the CIM test on a well characterised collection of *Escherichia coli* and *Klebsiella* spp.

Material/methods: Clinical *E. coli* (n=79) and *Klebsiella* spp. (n=328) which showed carbapenem resistance with VITEK 2 automated antimicrobial susceptibility test system (bioMerieux, France) were included in the study. The carbapenemase genes were investigated by an in-house multiplex polymerase chain reaction test designed for IMP, VIM, NDM-1, KPC, and OXA-48 genes. The Carba NP test was performed as per CLSI M100-S25 guideline. The CIM test was performed as described originally by van der Zwaluw et al. (PLoS One 2015;10). Briefly, a loopful of fresh colonies were suspended in 400 µL of sterile distilled water, 10 µg meropenem disk was immersed in the suspension and incubated for 2 hours at 35±2°C. At the end of the incubation period the disks were transferred from the suspension onto a Mueller Hinton agar (bioMérieux, France) which was inoculated with a 0.5 McFarland suspension of susceptible indicator strain *E. coli* ATCC 25922. Discs incubated in suspensions of carbapenemase-producing isolates were inactivated and thus allowed uninhibited growth of indicator strain, whereas discs incubated in suspensions without any carbapenemase activity yielded a clear inhibition zone.

Results: Among the study isolates, the OXA-48 positive isolates showed the highly discordant results; Carba NP test identified the carbapenemase activity in 201 of 249 (80.7%) OXA-48 positive isolates, whereas the CIM test identified only 95 isolates (38.2%) (Table 1). Presence of a metallo-beta-lactamase (MBL) together with OXA-48 increased the detection capacity of the tests, Carba NP detected 42/45 (93.3%) and CIM test detected 40/45 (88.9%) of such isolates. In the presence of MBL alone Carba NP detected 31/34 (91.2%) and CIM test detected 28/34 (82.4%) isolates correctly. The two KPC isolates were correctly identified by both methods. All together, the sensitivity and susceptibility of Carba NP test was calculated as 83.6% and 97.4%, respectively, and for the CIM test as 50.0% and 98.7%, respectively.

Conclusions: The Carba NP test was found superior to CIM test in regards to sensitivity and speed. For OXA-48 positive isolates which is the most prevalent carbapenemase type in Turkey, the Carba NP test showed low sensitivity (80.7%).

Table 1. Performances of Carba NP test and CIM for the investigation of carbapenemase activity in *Escherichia coli* and *Klebsiella* spp. isolates

		Carbapenemase Activity (n)	
Carbapenemase gene	**n**	**Carba NP**	**CIM**
IMP	2	2	2
KPC	2	2	2
NDM	32	29	26
OXA-48	249	201	95
OXA-48 + NDM-1	43	40	38
OXA-48 + IMP	1	1	1
OXA-48 + VIM	1	1	1
Negative	77	2	1