

TITLE: EVALUATION OF THE “RESIST-3 OKN” A NEW MULTIPLEX IMMUNOCHROMATOGRAPHIC ASSAY FOR THE RAPID DETECTION OF OXA-48, KPC AND NDM CARBAPENEMASES DIRECTLY FROM BACTERIAL COLONIES AND SPIKED BLOOD CULTURE

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ABSTRACT:

The emergence of carbapenemase producing *Enterobacteriales* (CPE) is a matter of public health concern and the rapid detection CPE is essential. The detection of carbapenemases is mainly based on molecular assays but, recently, an easy and rapid detection test for carbapenemases (OXA-48, KPC, and NDM) using nitrocellulose membrane has been developed using lateral flow immunoassay (LFIA) based on specific monoclonal antibodies. The aim of this study was to evaluate the multiplex “RESIST-3 OKN” directly from bacterial colonies as well as from spiked blood cultures. A total of 76 carbapenemase positive clinical isolates of *Enterobacteriales* were tested in pure culture: 29 KPC-producers, 21 NDM, 10 OXA-48-like, 3 GES, 1 IMP, 1 VIM, 5 NDM+OXA-48-like, 5 KPC+NDM, 1 KPC+GES. One *K.pneumoniae* ATCCBAA1705 (KPC+), 1 *E.coli* NCTC13476 (IMP+) and 3 clinical isolates of *Acinetobacter* spp (OXA-23 positive) were also tested. One *E. coli* ATCC25922 and 18 clinical isolates negative for carbapenemase genes were included as negative controls. The test directly from colonies was carried out according to the manufacturer’s protocol and the results were read within 15 min. The test directly from spiked blood culture was evaluated with 38 carbapenem resistant isolates: 11 KPC, 10 NDM, 8 OXA-48-like, 1 GES, 1 VIM, 1 IMP, 3 KPC+NDM, and 1 OXA-48-like+NDM. One *K.pneumoniae* ATCC BAA1705 (KPC positive) and 1 *E.coli* NCTC13476 (IMP-positive) were also evaluated. Eighteen clinical isolates negative for carbapenemase genes were included as negative controls. Positive results for both pure bacterial colonies and blood cultures were indicated by a dark pink band in the nitrocellulose membrane. All isolates with KPC, NDM and OXA-48-like from pure colonies presented positive results in the “RESIST-3 OKN” assay; 97% of the isolates presented positive results in less than 5 min. No false positive results were observed. For CPE directly from blood culture, the assay was positive for all KPC and OXA-48-like isolates. However, none NDM-positive isolates presented positive results. No false positive results were observed for the spiked blood cultures. These preliminary data indicate that “RESIST-3 OKN” assay is an accurate method for rapid identification of carbapenemases directly from pure colonies. This assay also detected KPC and OXA-48-like, but not NDM carbapenemase, directly from spiked blood cultures. Further analysis is required to identify possible inhibitors of NDM in the blood culture.

Keywords: Carbapenemases; Multiplex Immunochromatographic Assay; Rapid Diagnostics; RESIST-3 OKN

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