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Rapid Identification Of Oxa-48, Oxa-163 Subfamily And Kpc In Carbapenemase-producing Enterobacteriaceae

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Abstract:
Background: CPE are a leading cause of antibiotic resistance worldwide. OXA-48 and KPC are two carbapenemases expressed in CPE and their identification present a serious challenge for laboratories. Noteworthy, some allelic variants of OXA-48, such as OXA-163 which is highly prevalent in Argentina, show weak carbapenemase activity while it hydrolyses broad-spectrum cephalosporins. Definitive confirmation of OXA-48 variants currently relies on molecular assays and gene sequencing. We developed the OXA- 163/48 Duo K-SeT test, a new lateral flow assay that identifies OXA-48 and OXA-163-like carbapenemases, and we combined this test with the KPC identification in the OXA- 163/48 and KPC Trio K-SeT test.
Methods: Immunochromatographic sandwich tests were developed by using monoclonal antibodies in both capture (coating on membrane) and detection (coupling to colloidal gold). Tests are performed on bacterial colonies grown on solid medium after suspension in a specific buffer.Results:Two anti-OXA-48 antibodies were selected as specific capture reagents on two lines for the Duo test: (1) a first antibody directed against OXA-48 (but not OXA-163) and (2) another antibody directed against another epitope of OXA-48 and other variants (including OXA-163). A third antibody directed against all OXA-48 variants (including the OXA-163 subfamily) was chosen as a detection reagent. If the sample contains OXA-48, it will remain bound to the first capture antibody (anti-OXA-48). If the sample contains the OXA-163, it will not react with the first antibody and will bind to the second capture antibody (anti-OXA-163+48). This OXA-163/48 Duo K-SeT test allowed to accurately and rapidly identify (i) OXA-163 and other related variants (OXA-247 and -438) and (ii) OXA-48 and -181 from a panel of 40 positive clinical strains with 100% sensitivity. All 30 non-OXA-48 strains remained negative. The OXA-163/48 and KPC Trio K-SeT test allowed to detect KPC in all tested KPC positive strains.
Conclusions:
We developed the OXA-163/48 Duo and OXA-163/48 and KPC Trio K-SeT tests for the rapid identification of carbapenemases from the OXA-48 family, OXA-163 subfamily and KPC. The 100% performance of the tests will be further assessed on a larger panel of clinical bacterial isolates