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Reduction in the detection time of OXA-48, OXA-163 and KPC carbapenemases performed with a novel immunochromatographic lateral flow assay from 2-hour-old cultures (early cultures)

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Background: To prevent the spread of carbapenemase producers and define an appropriate empirical antimicrobial therapy, the rapid detection of carbapenemase-producing organisms has become imperative. We developed the OXA-163/48 and KPC Trio K-SeT test, a new lateral flow assay that identifies OXA-48 and OXA-163-like carbapenemases and combined this test with the identification of KPC by using monoclonal antibodies. This test has been validated on bacterial colonies grown on solid medium from 1 to 3-day-old cultures, with reported sensitivity and specificity of 100%. This study aims to reduce detection times by using 2-hour-old cultures (haze of bacterial growth) instead of overnight culture plates.

Material/methods: A total of 100 clinical gram-negative bacilli were included: 79 carbapenemase producers and 21 non-producers. PCR and DNA sequencing of entire *bla* alleles were considered the gold standard for beta-lactamase characterization. About 20 µl of an aqueous suspension containing 10^4 CFU/ml of each strain were spotted in Columbia agar with 5% sheep blood plates or chocolate agar plates or cystine-lactose-electrolyte-deficient (CLDE) agar plates and subsequently streaked with a sterile inoculation 1-µl loop. After 2 hours of incubation (2-hour-old cultures), bacterial suspension was made in 10 drops of LY-A buffer by streaking with a sterile inoculation 1-µl loop the area of the Petri plate where a haze of growth was observed. Subsequently, 3 drops of these homogenized solution were applied to the sample well of the Trio K-SeT. Tests were read by eye within 15 min. Plates were re-incubated to complete a total of 18 to 24 hours of incubation (1-day-old cultures) before being retested with the Trio K-SeT.

Results: The Trio K-SeT test allowed to accurately and rapidly identify from 2-hour-old cultures: (i) 29/29 OXA-163 and other related variants (OXA-247 and -438), (ii) 16/16 OXA-48 and other related variants (OXA-181, -232 and -244), (iii) 23/23 KPC-2 or KPC-3 variants, and (iv) 2/2 KPC-2 + OXA-163 dual producer strains (100% sensitivity). The Trio K-SeT yielded negative results from 2-hour-old cultures for 4/4 OXA carbapenemases typically found in Acinetobacter spp., 10/10 isolates with narrow-spectrum OXA-type enzymes, 9/9 isolates producing other major carbapenemase families (Sme, IMI, VIM, IMP, SPM, GES and NDM) and 7/7 isolates exhibiting reduced carbapenem susceptibility due to ESBL or AmpC production (100% specificity). Identical results were obtained with the 1-day-old cultures.

Conclusions: In this study we adapted the OXA-163/48 and KPC Trio K-SeT test for use with 2-hour-old cultures, improving the turnaround time for results, in comparison with traditional 1-day-old cultures, and maintained high test sensitivity and specificity. In clinical setting, utilizing younger cultures will allow incorporation of the K-SeT into a single day's workflow, facilitating, along with tests for rapid bacteria identification, timely patient care, antimicrobial stewardship, and infection control.