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Evaluation of two commercial assays for the rapid confirmation of OXA-48 like carbapenemases produced by *Klebsiella pneumoniae*

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Over the past decade, carbapenemase-producing Enterobacteriaceae (CPE) have emerged worldwide as significant pathogens (1). Rapid and accurate confirmation of carbapenemase production is essential to guide antimicrobial therapy and to ensure prompt implementation of infection control measures to prevent spread of CPE (2).

The aim of this study was to assess the performance of 2 commercially available assays for the rapid detection of CPE with OXA-48 like carbapenemases. These included a commercially-available PCR, i.e. the BD MAX CRE assay (Becton Dickinson, Canada), and a commercially available immunochromatographic assay, i.e. Resist-3 O.K.N K-SeT (Coris BioConcept, Belgium).

In a previous CPE surveillance program at the Hacettepe University Adult and Oncology Hospitals, Turkey, 279 isolates of *Klebsiella pneumoniae* were recovered from rectal screening swabs from unique patients. Two hundred and seventy consecutive isolates produced OXA-48 like carbapenemases and one produced IMP carbapenemase, as confirmed by in-house PCR methods (3). Species identification was confirmed by MALDI-TOF MS (Bruker, Coventry, UK). One hundred and ninety-nine of these isolates were selected for inclusion in this study including 192 isolates with OXA-48 like carbapenemases and 7 isolates without any carbapenemase. The MIC of carbapenems for these isolates ranged widely from <1 to >32 mg/L (3).

Prior to testing for carbapenemase activity, each isolate was retrieved from storage at -80 °C and subcultured on Mueller-Hinton agar (Oxoid) with incubation for 18 h at 37 °C in air. Each isolate was tested with the 2 commercial assays in exact accordance with the manufacturer's instructions. The BD MAX CRE assay is a research use only automated PCR assay allowing detection

and distinction of the 3 most commonly encountered carbapenemase genes: KPC, OXA-48, and NDM. The assay automates sample extraction, amplification, and detection by using real-time PCR with a total running time of 90–120 min (depending on batch size). There is sample processing control in the extraction tube that undergoes the extraction, concentration, and amplification steps to monitor the assay inhibition as well as process inefficiency due to instrument or reagent failure.

The RESIST-3 O.K.N. K-SeT is an immunochromatographic cartridge assay that employs a membrane technology with colloidal gold nanoparticles. This test also allows detection and distinction of KPC, NDM, and OXA-48 like carbapenemases from a single colony of Enterobacteriaceae. The result is visible within 15 min in the form of red lines on the strip. For both assays, the preanalytical handling time was 5–7 min.

In blind testing, both the BD MAX CRE and the RESIST-3 O.K.N. K-SeT successfully detected OXA-48 like carbapenemases in 192 isolates and gave negative results for the 7 negative controls. No other carbapenemases were detected and no repeat tests were necessary. Thus the sensitivity and specificity of the 2 assays were 100%.

No instrumentation is required for the RESIST-3 O.K.N. K-SeT and a result can be generated in around 20 min, compared with at least 95 min for the BD MAX CRE assay (including handling time). The cost of the BD MAX assay is approximately 28.7 Euros per sample compared with 10.5 Euros for the RESIST-3 O.K.N. K-SeT.

A range of laboratory assays has been developed for detection of carbapenemases that can be applied to bacterial colonies. These include matrix-assisted laser-desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), immunochromatographic assays, susceptibility tests that employ β -lactamase inhibitors

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and chromogenic tests such as the CARBA-NP test (4–6). Alternatively, the presence of carbapenemases may be inferred by detection of target genes using molecular assays, e.g., using PCR or microarrays (7). OXA-48 carbapenemase was first reported in *Klebsiella pneumoniae* isolated in İstanbul in 2001 (8). Since then, OXA-48 has become the predominant carbapenemase type in Turkey and many European countries. In some phenotypic assays, detection of some OXA-48 producers has proven to be problematic due to a low level of carbapenemase activity (resulting in low levels of resistance to carbapenems) and the lack of a specific inhibitor to assist detection (9). Recent epidemiological studies have shown that OXA-48 is the most prevalent carbapenemase in many European countries such as France, Spain, and Belgium and also in the Middle East, North Africa, and Turkey (10). In the present study, we showed that both commercial assays are highly effective for detection of OXA-48 like carbapenemase producers and that either assay could constitute a suitable first line test for investigation of suspected CPE. A limitation of both tests is their inability to detect VIM carbapenemase and, to a lesser extent, IMP carbapenemase, and a negative test might therefore necessitate a second test to rule out the presence of these

metalloenzymes. In a setting such as Turkey, where OXA-48 is by far the dominant carbapenemase enzyme, this is an acceptable limitation. High levels of performance were also reported by Glupczynski et al. (11), who challenged the assay with a well-defined collection of 112 carbapenem-resistant Enterobacteriaceae including a range of species with OXA-48-like, NDM, and KPC enzymes.

When comparing the 2 commercial assays there are several advantages of the RESIST-3 O.K.N. K-SeT. No instrumentation is required for the RESIST-3 O.K.N. K-SeT and a result can be generated in around 20 min, compared with at least 95 min for the BD MAX CRE assay. The cost of the BD MAX assay is approximately 28.7 Euros per sample compared with 10.5 Euros for the RESIST-3 O.K.N. K-SeT. Compared with phenotypic assays that detect general carbapenemase activity (e.g., the modified Hodge test, the CARBA NP test, or MALDI-TOF MS), both assays have the inherent limitation that some carbapenemases will not be detected, but they have the advantage that they allow for differentiation of the 3 major types of carbapenemases. We conclude that both assays are convenient, accurate, and rapid ‘first line’ tests for confirmation of OXA-48 like carbapenemase producers.

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