Objectives: Carbapenemases (CARBA) are reported increasingly in Gram-negative bacilli (GNB) and represent an emerging public-health concern. Laboratory detection of CARBA-producers remains a challenge for the microbiology laboratory and is important to avoid clinical failure due to inappropriate antimicrobial therapy and to prevent nosocomial outbreaks. We evaluated a new molecular test for detection of NDM, KPC, OXA-48, VIM and IMP carbapenemase producers. Methods: We evaluated the « Check-MDR Carba » test (Check-Points, Wageningen, Netherlands) that is based on specific molecular recognition of blaNDM, blaKPC, blaOXA-48, blaVIM and blaIMP by DNA probe ligation followed by real-time PCR detection. 183 well-characterized Gram-negative rods (Enterobacteriaceae, Pseudomonas sp., Acinetobacter sp., Aeromonas sp. and Alcaligenes sp.) isolates possessing different bla genes were tested. Several wild-type isolates or isolates harbouring other ß-lactamase genes were used as controls. Total DNAs were extracted using NucliSens® easyMAG™ automated system (bioMerieux) and real time PCR was performed on a ABI 7500 real-time PCR instrument (Applied Biosystems). Results: The “Check-MDR Carba” system detected correctly the five carbapenemase gene families tested using cultured strains. Specificities and sensitivities of 100% were recorded for the blaKPC, blaVIM, blaIMP, blaNDM, and blaOXA-48 genes. All positive isolates gave Ct values between 21.0 and 32.0 as recommended by the manufacturer for results interpretation. No isolate should be repeated because of inconclusive result. The test detects all known variants of KPC, NDM, OXA-48 and VIM, except VIM-7, a rare variant only found in P. aeruginosa. IMP is the most diverse carbapenemase gene family, and the test detects the clinically most important IMP-variants in Enterobacteriaceae, including IMP-1, -3, -4, -5, -6, -7, -8, -10 and -13. Conclusion: Check-MDR Carba generates definitive results within 4.5 hours, compared to the 24 hours necessary for analysis with conventional phenotypic methods. This molecular test rapidly differentiates carbapenem-resistant isolates by mean of the production of a carbapenemase from those associated to non-ß-lactamase-mediated mechanisms of resistance to carbapenems.