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Rapid Detection of MBL Genes by SYBR Green Based Real-Time PCR

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Abstract : Objectives: To develop SYBR green based real-time PCR assay to detect carbapenemases (NDM, IMP) genes in E. coli. Methods: A total of 40 E. coli from stool samples were tested. Six were previously characterized as resistant to carbapenems and documented by PCR. The remaining 34 isolates previously tested susceptible to carbapenems and were negative for these genes. Bacterial RNA was extracted using manual method. The real-time PCR was performed using the Light Cycler III 480 instrument (Roche) and specific primers for each carbapenemase target were used. Results: Each one of the two carbapenemase gene tested presented a different melting curve after PCR amplification. The melting temperature (Tm) analysis of the amplicons identified was as follows: blaIMP type (Tm 82.18°C), blaNDM-1 (Tm 78.8°C). No amplification was detected among the negative samples. The results showed 100% concordance with the genotypes previously identified. Conclusions: The new assay was able to detect the presence of two different carbapenemase gene type by real-time PCR.

Keywords : resistance, b-lactamases, E. coli, real-time PCR
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