TITLE: EVALUATION OF FILTER PAPER TO TRANSPORT BACTERIA FOR CARBAPENEMASE GENES DETECTION USING PCR BY HIGH MELTING RESOLUTION

AUTHORS: CARNEIRO, M. S.[1,2]; CRISPIM, M. N.[1]; WINK, P. L.[1,2]; ALMEIDA, E. K.[1]; BARTH, A. L.[1,2]

INSTITUTION: [1]LABORATÓRIO DE PESQUISA EM RESISTÊNCIA BACTERIANA (LABRESIS), CENTRO DE PESQUISA EXPERIMENTAL (CPE), HOSPITAL DE CLÍNICAS DE PORTO ALEGRE (HCPA), (R. RAMIRO BARCELOS, 2350, SANTA CECILIA, CEP 90035-903, PORTO ALEGRE – RS, BRAZIL). [2]PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS (PPGCF), FACULDADE DE FARMÁCIA, UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL (UFRGS), (AV. PAULO GAMA, 110 - FARROUPILHA, PORTO ALEGRE – RS, BRAZIL)

ABSTRACT:
Infections caused by resistant microorganisms are a complex global public health challenge. One important way to combat the increase of resistance is the use of multiple strategies, as the development of more modern and fast techniques in the detection of resistance. The use of filter paper has already been applied for the transport of biological samples, but not bacteria, and has proved to be efficient. This study aimed to evaluate the transport of inactivated bacteria impregnated in filter paper under normal environmental conditions for further analysis of carbapenemase genes using a High Melting Resolution (HRM) PCR with specific primers. Isolates of *Enterobacterales* with characterized carbapenemase genes (bla_{KPC}, bla_{NDM}, $bla_{OXA48\text{-}like}$, $bla_{KPC+NDM}$ coproducers) were evaluated. The isolates were subcultured onto Mueller Hinton (MH) agar and incubated at 36ºC +/- 1ºC for 18 to 24 hours. Bacterial growth on MH (full loop of 10 µL) was impregnated in a filter paper. The filter paper was left at room temperature for 2 days in order to simulate the transport time. The filter paper disks were placed in sterile eppendorfs and the DNA was extracted by thermal lysis, followed by quantification on NanoDrop Nucleic Acid Quantification (Thermo Fisher Scientific). We evaluated a total of 48 carbapenemase positive clinical isolates impregnated in the filter paper disks and the carbapenemase genes were correctly identified in 47 isolates (97.9%). Only one isolate bla_{NDM} presented negative results for carbapenemase genes. Noteworthy, the three isolates carbapenemase negative presented negative results after being impregnated in the filter paper. Our preliminary results indicated that it is possible to detect carbapenemase genes from bacteria impregnated in filter paper. Thus, the filter paper can be considered as an alternative to transport bacterial biomass in order to evaluate the presence of carbapenemase genes by PCR.

Keywords: filter paper, bacteria transportation, High Melting Resolution

Development Agency: Instituto Nacional de Pesquisa em Resistência Antimicrobiana (INPRA)