TITLE: DEVELOPMENT AND STANDARDIZATION OF THE ELISA TEST FOR DETECTION OF KPC IN BACTERIAL CLINICAL ISOLATES

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ABSTRACT

The association of third-generation cephalosporins with new β-lactamase inhibitors has become a new ally in the treatment of serious infections caused by gram-negative bacteria producing class A and D carbapenemases. Currently, few automated tests are available to differentiate between these carbapenemases in clinical microbiology laboratories. We aimed to standardize a new enzyme-linked immunosorbent assay (ELISA) for the detection of KPC in bacterial isolates. The bla_{KPC-2} gene was cloned into pET26b+ and expressed in *Escherichia coli* BL21D3 to obtain the KPC-2 protein and purified using the AKTA system. The purified protein was used for immunization of Balb/c mice and New Zealand rabbits, using aluminum hydroxide as adjuvant. The animals' serum was extracted, and the antibodies were purified with the G-Sepharose column using the AKTA equipment. Block titling was used to assess competition among epitopes and determine the concentration to be used to standardizing the ELISA test. Four different methods of preparation of the isolates were evaluated: 1. Bacterial colony obtained directly from the Müeller Hinton (MH) agar (BC); 2. Bacterial growth in MH broth (BG-18h-2) after two incubation of 18h; 3. Bacterial growth in MH broth until reaching an optical density of 1.0 (BG-DO); and 4. Bacterial growth in MH broth after 18h of incubation (BG-18h). The sensitivity and specificity of the ELISA test were evaluated using four *Klebsiella pneumoniae* isolates producing KPC-2, three *K. pneumoniae* producing BKC-1, one strain of Pseudomonas aeruginosa producing SPM-1, one strain of *E. coli* producing NDM-1, a *P. aeruginosa* strain producing VIM-1, and a *P. aeruginosa* strain producing GES-5, and the ATCC 25922 and 700603 (SHV-18) standard strains of *E. coli* and *K. pneumoniae*, respectively. Sensitivity and specificity were determined by the ROC curve and Student's t-test was used to assess the statistical difference between positive and negative cases for each method. The BC, BG, BG-DO, and BG-18h methods had a sensitivity of 75%, 100%, 100%, and 100% and specificity of 77.8%, 88.9%, 100%, and 100%, respectively. The BC method did not show any statistical difference between isolates producing and not producing carbapenemases (p: 0.1388). On the other hand, the BG-DO, and BG-18h showed better discriminatory power when compared to other bacterial cultivation methods.

Keywords: bacterial resistance, diagnosis, carbapenemase