TITLE: COMPARISION OF PHENOTYPIC TECHNIQUES FOR DETECTION OF EXTENDED-SPECTRUM β-LACTAMASES AND CARBAPENEMASES PRODUCED BY GRAM-NEGATIVE BACTERIA IN MICROBIOLOGICAL PRATICE

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ABSTRACT: Enterobacteriaceae and Gram-negative non-fermentative bacilli, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* belong to the most feared group of bacterial pathogens, recently listed among the 12 most dangerous bacteria to human health according to the World Health Organization. The production of extended-spectrum β-lactamases (ESBL) and carbapenemases by those organisms can be associated with failure of antibiotic therapy and related morbidity and mortality. Although advances in the description of methods that perform early and correct detection of these enzymes have been made in recent years, the choice of detection methodology remains a difficult problem for microbiologists in the clinical routine. The aim of this study was to compare the performance of selected phenotypic methods, including methodologies recently described and those that are already well established and applied in the routine. The evaluation was performed with Gram-negative bacteria isolated from blood cultures collected from patients hospitalized at Hospital São Rafael (Salvador, Bahia) from March 2015 to March 2016. Positive blood cultures were detected by BacT/ALERT (bioMeriéux) and the microbial identification were performed by MALDI-TOF MS (bioMeriéux). The antimicrobial susceptibility testing was performed by VITEK 2 (bioMeriéux), and the identification of ESBL producing Enterobacteriaceae were performed by VITEK's *Advanced Expert System* (AES) and by the disk approximation test as described by CLSI. Isolates resistant to at least one carbapenem were analyzed by the following phenotypic approaches: Modified Hodge Test (MHT), Carbapenemase Inactivation Method (CIM), Indirect Carbapenemase Test (ICT) - with and without addition of Triton-X, RAPIDEC CARBA NP, Blue-CARBA and the test recommended by ANVISA Technical Note 01/2013 for the detection of carbapenemases. To confirm the presence of ESBL and carbapenemases, genes that decode those enzymes were screened by PCR (^{bla}TEM, ^{bla}SHV, ^{bla}OXA-1-like, ^{bla}CTX-M, ^{bla}GES, ^{bla}OXA-48-like, ^{bla}KPC, ^{bla}VIM, ^{bla}IMP, ^{bla}NDM, ^{bla}OXA-23-like). During the study period 170 isolates were recovered from blood cultures, of which 137 (80%) were Enterobacteriaceae and 29 (16.4%) were non-fermentative bacilli. The ESBL phenotype was detected in 36 Enterobacteriaceae by VITEK-2, whereas disk approximation test detected this phenotype in 40 isolates. Using the molecular methods as gold standard, the sensitivity of VITEK-2 and manual test were 90.9% and 85.7%, respectively, and the agreement between the two methods was 75%. Among the organisms investigated, 18 showed resistance to at least one carbapenem. The most satisfactory performance test was the MHT with sensitivity, specificity and accuracy of 83.33%, 91.67% and 88.89%, respectively. However, when considering only the sensitivity parameter, CIM and ICT (with addition of Triton-X) reached values of 100%. The ^{bla}KPC (*n*=5) gene was found in *Klebsiella pneumoniae* (*n*=4) and *Escherichia coli* (*n*=1), and the sensitivity of all methods here tested on the detection of this enzyme was 100%. Our results, suggest that, indeed, the detection of ESBL production by VITEK-2 is a better choice, since the sensitivity of this technique is above 90%. However, the disk approximation test showed a concordance of 75% with the automated method, remaining as a viable alternative in clinical laboratories in which the implementation of an automated routine is not possible. Regarding the detection of carbapenemases, the MHT remains a good choice for detection, being the most accurate method. When considering KPC enzymes, all methods tested in this work shown suitable and highly sensitive alternatives.

Keywords: Phenotypic tests, broad-spectrum b-lactamases, Gram-negative bacteria

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