TITLE: COMPARISON BETWEEN PHENOTYPIC IDENTIFICATION BY BIOCHEMICAL TESTS AND DNA SEQUENCING IN BETA-LACTAMASES PRODUCING STRAINS.

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ABSTRACT:

Gram-negative bacteria producing Extended Spectrum Beta-Lactamases (ESBL) are able to hydrolyze the beta-lactam ring of the chemical structure of penicillins, cephalosporins and monobactams. These antimicrobials have been the first choice in the treatment of bacterial infections. Failure to treat worsens the patient's condition, leading to a state of morbidity and mortality. The identification of the community of ESBL bacteria provides a better guidance in the treatment of the patient. Manual biochemical tests for phenotypic identification used in a public diagnostic laboratory have been restricted to identify all species. Thus, through the sequencing of the RNAr 16S gene, reference method for bacterial identification, this research aimed to determine ESBL Gram-negative bacteria species from biological samples of patients from public hospitals in the state of Roraima, in order to compare the phenotypic and molecular results. The clinical isolates (25 samples) were kindly provided by the Central Laboratory of Roraima (LACEN-RR). The biological samples were seeded on MacConkey agar and incubated at 37 ° C for 24h. The disc diffusion antibiogram was then performed on Muller-Hinton agar for the identification of ESBL by the disc-approximation technique with Amoxicillin associated with Clavulanic Acid in the center and surrounded by discs of Aztreonam, Cefotaxime, Ceftazidime and Cefepime. Concomitantly, phenotypic identification was performed by the biochemical series. The ESBL-positive isolates in the phenotypic test were subjected to DNA extraction by thermal shock. The Polymerase Chain Reaction (PCR) was performed for the 16S RNAr gene, with primers 27F and 1492R, generating a fragment of 1500bp. The strain *Klebsiella pneumoniae* ATCC 700603 was used as a positive control for ESBL. The amplified product was subjected to automatic sequencing by DNA strand disruption. The consensus sequence was aligned with GenBank sequences via the BLAST platform by the Geneious software, for identification of the species. The sequencing results showed the identity of twenty five bacterial species, of which twelve species were convergent with the biochemical identification and thirteen were divergent. This divergence may be related to the non-correct isolation of the colonies, scarcity of culture media to increase the panel of species, rendering inconclusive results in the biochemical series. The profile of strains related to pathogenicity is extremely necessary due to the intrinsic resistance present in several species such as *Klebsiella pneumoniae*, among others. This research showed that the phenotypic profile by biochemical methods did not have results that match the species diagnosed.

Keywords: biochemical tests, molecular analysis, RNAr 16S

Development Agency: Instituto Federal de Educação, Ciência e Tecnologia de Roraima – Edital PIPAD