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Polyphasic identification of clinical multidrug-resistant bacteria isolated from blood cultures

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Bloodstream infections of hospitalised patients caused by multidrug-resistant bacteria, that include methicillin-resistant *Staphylococcus aureus* (MRSA), are a significant cause of morbidity and mortality. Multidrug-resistant bacteria have emerged as a serious public health problem worldwide and require consistent and intensified surveillance efforts.

The aim of the present work was to identify a set of 282 multidrug-resistant bacteria isolates recovered from blood cultures at The University Hospital Cassiano Antônio de Moraes (HUCAM, Vitória, ES, Brazil). For bacterial identification a polyphasic approach, including classic phenotyping analyses, MALDI-TOF MS and molecular biology methods, was employed.

From January to December 2011 blood cultures from bloodstream infections of 3214 patients were analysed. After incubation in the Bactec 9240 system at 35°C, a total of 471 isolates were positive. Out of these isolates, 282 (60%) were identified at species level through a polyphasic approach that included: VITEK[®]2 Systems, Manual Conventional Phenotypic Methods and MALDI-TOF MS. Furthermore, isolates that showed limitation on the protein extraction for proteomics analysis by MALDI-TOF MS and that produced discordant proteomic profile by this method were analysed by molecular biology using PFGE to the genetic polymorphism determination.

Comparing MALDI-TOF MS bacterial identification results with those obtained from the other methods applied a high level of accuracy (96%) was obtained. Moreover, in the Enterobacteriaceae group blaTEM gene was the most prevalent ESBL gene (58%) followed by blaCTX-M (42%) and blaSHV (25%). blaVIM and blaKPC genes were observed only for two strains of *K. pneumoniae*. Carbapenemase activity was not detected in *P. aeruginosa*. blaOXA-23 gene was found in 92% (11/12) of the *A. baumannii* strains and blaOXA-58 only for 1 strain of this species. It was observed 100% of vanA gene in *E. faecium* VRE (n=12) and 100% of vanC gene in *E. gallinarium*. The presence mecA gene which is usually found in MRSA was observed in 90% (35/39) of all *Staphylococcus aureus* strains analysed. All identified strains were preserved in a deep freezer at -80 °C by the Culture Collection of Bacterial Reference Strains (CCBR) of HUCAM (CCBR-HUCAM), hosted by the Department of Microbiology.

In conclusion, the 282 multidrug-resistant strains are now well identified and characterised with relevant associated information becoming an important asset to the CCBR-HUCAM.