Identification of Uncommon Microbial Species by MALDI-TOF MS and 16S rRNA Sequence analysis: Data from a Clinical Microbiology Laboratory

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Background: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF-MS) has revolutionized the practice of pathogens identification in clinical microbiology laboratories. Recently, this technology has been reported for new applications as: bacterial typing and direct antibiotic susceptibility testing of clinical samples in a simple, fast and low-cost way, which may further contribute to improve microbial diagnostic. The aim of this study was to describe and alert regarding the identification and emergence of rare and uncommon microorganisms causing opportunistic infections. **Methods:** From March 2014 to January 2020, the clinical microbiology section of AFIP Laboratory identified several unusual bacterial species recovered from blood, peritoneal fluid, broncho-alveolar lavage, respiratory tract, wound and urine. All the clinical samples were cultivated in Tryptcase Soy with 5% of sheep blood, MacConckey and Chocolate agar plates, and then incubated in aerobic and anaerobic atmospheres for 24-48h. All the isolates were identified by MALDI-TOF MS (Vitek-MS® System) using MYLA software (bioMerieux, Marcy-I'Etoile, France), according to the manufacture's recommendations. For specific samples, a second attempt to identify the infectious agent was performed using Sanger's sequencing methodology with universal primers for the 16S region of the rRNA. The amplified product was sequenced using a BigDye kit v3.1 and compared with sequences available in the GenBank data bases. **Results:** Thirty-three unusual species were identified in this period of analyses. Out of these, 02 were classified as gram-positive (*Cellulosimicrobium cellulans* and *Weissella confusa*) and 31 as gram-negative bacterial, as following: *Aggregatibacter aphrophilus, Eikenella corrodens, Empedobacter brevis, Kodamaea ohmeri, Cronobacter sakazakii, Achromobacter denitrificans, Achromobacter xylosoxidans, Aeromonas hidrofila, Aeromonas sobria, Bordetella bronchiseptica, Chryseobacterium gleum, Chryseobacterium indologenes, Comamonas aquática, Cupriavidus metallidurans, Delftia acidovorans, Edwardsiella tarda, Leclercia adecarboxylata, Ochrobactrum anthropi, Oligella urethralis, Porphyromonas pogonae, Pandoraea apista, Pandoraea pnomenusa, Pasteurella multocida, Raoultella ornithinolytica, Roseomonas gilardii, Shewanella algae, Sphingomonas paucimobilis, Brevundimonas diminuta/vesicularis, Elizabethkingia meningoseptica, Haematobacter massiliensis* and *Sphingobacterium thalpophilum.* The last five gram-negative bacilli are classified as non-fermenting bacteria and the most prevalent infections were bloodstream and wound. **Conclusions:** The microbiological diagnostic by MALDI-TOF MS had significantly improved the pathogens identification, especially for those not easily identified by conventional phenotypic methods as non-fermentative gram-negative bacilli and anaerobes. However, in some specific cases where the bacterial species is not yet available in the Myla system database the sequencing of the 16S rRNA portion the can be applied.