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MALDI-TOF Mass Spectrometry analysis for the differentiation of the pathogenic intestinal amoeba *Entamoeba histolytica* from the non-pathogenic *Entamoeba dispar* isolated from biological samples

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INTRODUCTION: The classification of the pathogenic species *E. histolytica* and the non-pathogenic species *E. dispar* as separate but not microscopically distinguishable species has led the WHO to recommend the development and application of improved methods for specific diagnosis of *E. histolytica* infection. The aim of this study was to investigate the potential role of MALDI-TOF Mass Spectrometry (MS) as a tool for the rapid identification of specific biomarkers suitable for the identification and differentiation of *E. histolytica* and *E. dispar*. This was done using amoebas from culture obtained from clinical samples in order to evaluate its application in the routinely diagnostic practice.

MATERIALS AND METHODS: MALDI-TOF MS was applied to *E. histolytica* strain 8026 and *E. dispar* strain 1557, isolated from faeces in our laboratory by using Robinson's medium monoxenic cultures (containing *Escherichia coli* ATCC 35218) and differentiated by molecular methods. Aliquots of cultures of these strains were submitted to formic acid/acetonitrile protein extraction and the protein extracts were analyzed by MicroFlex LT mass spectrometer (Bruker Daltonics, Germany supplied by Becton Dickinson, Italy). For the acquisition of the spectra the MBT_Standard method in manual mode acquisition was used with overall 520 laser-shots by 40 shot-steps each in different points of the sample. The spectra obtained for each of the investigated strains and for Robinson's medium with and without *E. coli* were analyzed by FlexAnalysis software and by the statistical software ClinProTools.

RESULTS: The obtained spectra are shown in Fig. 1a. The statistical tool PCA was applied to the analyzed datasets to visualize the homogeneity and the heterogeneity of the protein spectra. In Fig. 1b plot of the spectra in a three-dimensional space is reported showing the replicates of the spectra of *E. histolytica* strain 8026 and *E. dispar* strain 1557 and those of Robinson's medium with and without *E. coli* as four separate clusters. The corresponding average spectra were analyzed by ClinProTools software between 4,500 and 10,000 Da, which included the major differences between the protein profiles of the two amebic strains. Five discriminating peaks between *E. histolytica* and *E. dispar* strains were found: 2 peaks (8,250 and 8,300 Da) were found only in the *E. histolytica* protein profile and 3 (5,400, 5,500 and 5,545 Da) only in the *E. dispar* protein profile (Fig. 1c).

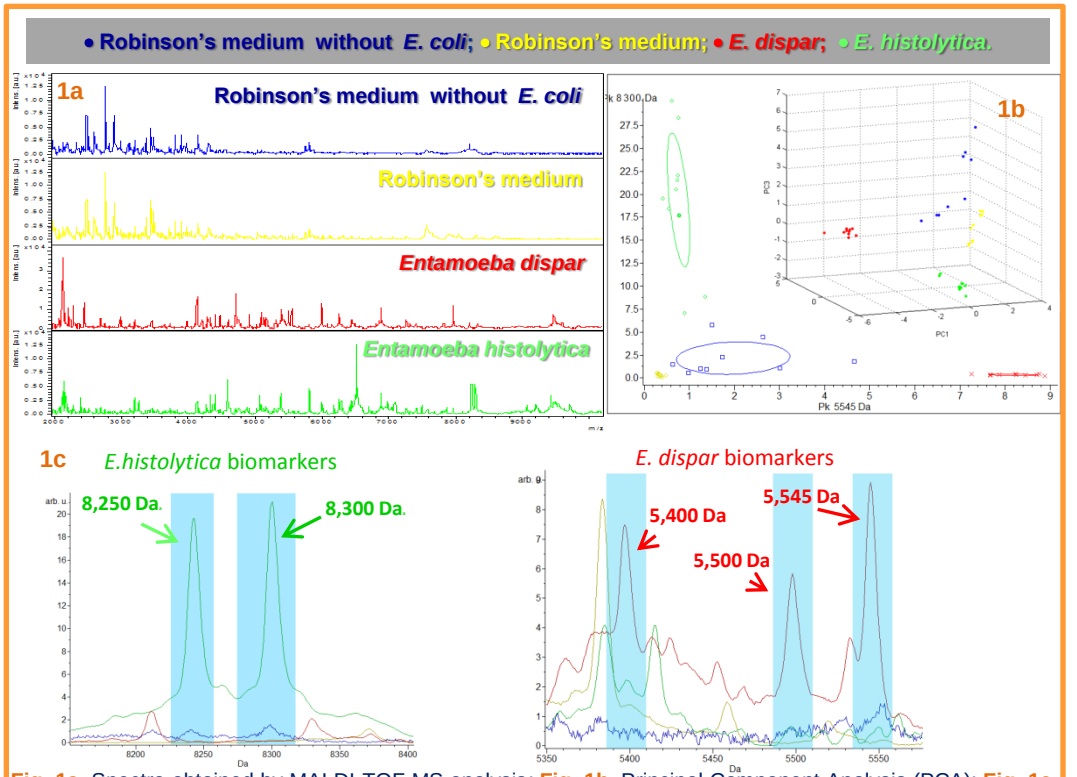

Fig. 1a. Spectra obtained by MALDI-TOF MS analysis; **Fig. 1b.** Principal Component Analysis (PCA); **Fig. 1c.** m/z window bins with the details of 5 discriminating peaks. Robinson's medium without *E. coli* is in blue; Robinson's medium is in yellow; *E. dispar* is in red; *E. histolytica* is in green.

CONCLUSION: The discrimination between *E. dispar* and *E. histolytica*, the causative agent of invasive amoebiasis, is an important goal of the clinical parasitology laboratory. In particular, a new challenge is to overcome the limits of molecular methods, being those cumbersomes and expensives. The preliminary results obtained in this study are very encouraging because in our experience they showed the ability of MALDI-TOF MS to detect different biomarkers for the pathogenic species *E. histolytica* and the non-pathogenic species *E. dispar*, respectively, distinguishable only by molecular methods. Being MALDI-TOF MS more rapid and less expensive than molecular methods, it could be used for the differentiation of *E. histolytica* and *E. dispar* field isolates. Future perspectives of our study will concern the analysis by MALDI-TOF MS of additional *E. histolytica* and *E. dispar* strains in order to assess the usefulness of this method in the differentiation of these two species.

References: 1) Calderaro A, Gorrini C, Bommezzadri S, Piccolo G, Dettori G, Chezzi C. (2006) *Entamoeba histolytica* and *Entamoeba dispar*: comparison of two PCR assays for diagnosis in a non-endemic setting. Trans R Soc Trop Med Hyg 100(5):450-7; 2) Calderaro A, Piccolo G, Montecchini S, Buttrini M, Gorrini C, Rossi S, Arcangeletti MC, De Conto F, Medici MC, Chezzi C. (2013) MALDI-TOF MS analysis of human and animal *Brachyspira* species and benefits of database extension. J Proteom 78:273-280; 3) Calderaro A, Gorrini C, Piccolo G, Montecchini S, Buttrini M, Rossi S, Piergianni M, Arcangeletti MC, De Conto F, Chezzi C, Medici MC. (2014) Identification of *Borrelia* species after creation of an in-house MALDI-TOF MS Database. PLoS One 12;9(2):e88895.