

Rapid Identification of Pathogenic Neisseria by Atmospheric Pressure MALDI-MS/MS

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Introduction:

Neisseria meningitidis (Nm) and *Neisseria gonorrhoeae* (Ng) are closely related and important human pathogens of Neisseriaceae family. Meningococcal meningitis caused by Nm and pelvic inflammatory disease caused by Ng are serious diseases of public health concern. We have used atmospheric pressure MALDI-MS followed by selective ion tandem mass spectrometry and MASCOT proteome data base search analysis to rapidly identify pathogenic Neisseria. Heat inactivated whole bacterial cell suspensions representing meningococcal serogroups A, B, C, W135 and Y and strains of gonococci were used for selective on-probe protein extraction by acid and base protocols. Three Neisseria specific proteins were identified by this method.

Methods

One micro liter of heat inactivated aqueous cell suspensions (10^3 to 10^4 cell/ μ l) were processed on the C-18 coated target plate at 50° C. Chemical processing of samples include extraction of species-specific proteins either by 10% TFA (acid-protocol) or 50% NH₄OH (base-protocol) followed by on-probe tryptic digestion, sample clean up and co-crystallization with MALDI matrix (α -Cyano-4-hydroxycinnamic acid), all steps being done *in-situ* on the plate. Species-specific biomarker peptides were identified by AP-MALDI MS/MS measurements combined with MASCOT search against NCBI database. AP-MALDI mass spectra were recorded on an LCQ-Deca XP ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) with an atmospheric pressure MALDI ion source (Mass Tech, Columbia, MD, USA).

Results

We have successfully tested, Nm clinical isolate strain numbers F8238 (serogroup A) H44/76 (serogroup B), 2120 (serogroup C), S1975 (serogroup Y) and S4383 (serogroup W135) and Ng strains GC192, GCMS11, GC186 using this method. Limit of detection (LOD) was determined ($\sim 10^3$ cells per sample) using serial dilutions and plate counts of cell suspensions before heat inactivation (at 60°C for 45 min). Amino acid sequences derived from at least three protonated peptide masses of m/z 1743.8, 1893.8 and 1946.8 identified corresponding proteins as *Neisserial* acyl carrier protein, conserved hypothetical protein and putative DNA binding protein respectively by atmospheric pressure (AP) matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) followed by selected ion tandem mass spectrometry (MS/MS) and MASCOT proteome database search analysis.

Novel aspect:

This is the first report on rapid identification of pathogenic *Neisseria* using advanced mass spectrometry.