Detection of Macrolide and Tetracycline resistance biomarkers in clinical isolates of *Neisseria gonorrhoeae* by Mass Spectrometry

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INTRODUCTION: *Neisseria gonorrhoeae* (NG) is the Gram-negative diplococci responsible for gonorrhoea. As most of the NG clinical isolates have developed resistance to at list one of the antibiotic drugs prescribed to this sexually transmitted disease, including tetracyclines (T) and macrolides (M), the microorganism susceptibility pattern should determine the choice of therapy[1,2]. Several studies have shown that MALDI-ToF significantly accelerates the resistance phenotyping compared to the commonly used routine antibiotic susceptibility tests [3].

1 OBJECTIVES

To develop a shorter and clinically applicable protocol that allows resistance phenotyping to M and T in NG clinical isolates using MALDI-ToF technology.

To show that the Mass Spectrometry (MS) data which will be obtained contain strong signal for the clustering of M and T antibiotics resistant (R) and susceptible (S) NG clinical isolates.

To applicate supervised learning to extract and verify the important features leading to the clustering.

(Future perspective) To reprocess the false negatives by Tandem Mass Spectrometry (MS/MS) providing an adequate dataset for the development of further studies.

2 MATERIALS AND METHODS

The study samples will be pre-processed and classified into two experimental groups, as illustrated in the following diagram (**See diagram 1**).

BIOMARKERS DETECTION

The spectrometric analysis will be carried out via MALDI-Tof system (Reflectron mode). The generated data will be imported in two different datasets (T = D1 and M = D2) using the "Maldiquant" package. The R and S isolates spectra for the studied antibiotics will be aligned between each other. An intensity-based feature matrix for the signature detected peaks will be performed.

For matching the peaks of interest with their respective proteins, their m/z value will be compared against the SwissProt/TrEMBL database by manual search.

3 MATERIALS AND METHODS

RANDOM FOREST MODEL

D1 and D2 will be used as learning data for the detection of T and M resistance respectively. Spectrums proceeding from new clinical isolates will be pre-processed as the ones belonging to datasets 1-2 and all together will be used for the building of a Random Forest model for testing, serving as training data.

4 EXPECTED RESULTS

BIOMARKERS DETECTION

Ribosome-based resistance (RBS) biomarkers will be detected within 12.000 m/z range [4]. Signature peaks correlated with little ribosomal subunit proteins will be emplaced near 9.500 m/z (D1).

Variants of this peaks may be seen in the same m/z range in D2.
Between 35.000 – 37.000 m/z, porin B defects could be measured. The differential peak might be seen in both datasets. We can expect the peaks related to RND pumps near 40.000 m/z (D2). Other pumps such as acrEF-tolC, mexCD-oprJ, and mtrCDE can also be emplaced there.

RANDOM FOREST MODEL

Random Forest should perform a binary discriminant analysis in which samples will be classified into: M and T (R)- isolates, M and T (S)-isolates, M (S) and T (R) isolates and *vice versa*. True positive results should be treated with cefixime and ceftriaxone, whereas true negative results with azithromycin and T. False positive results will not be detectable, whereas false negative results should be processed by MS/MS.

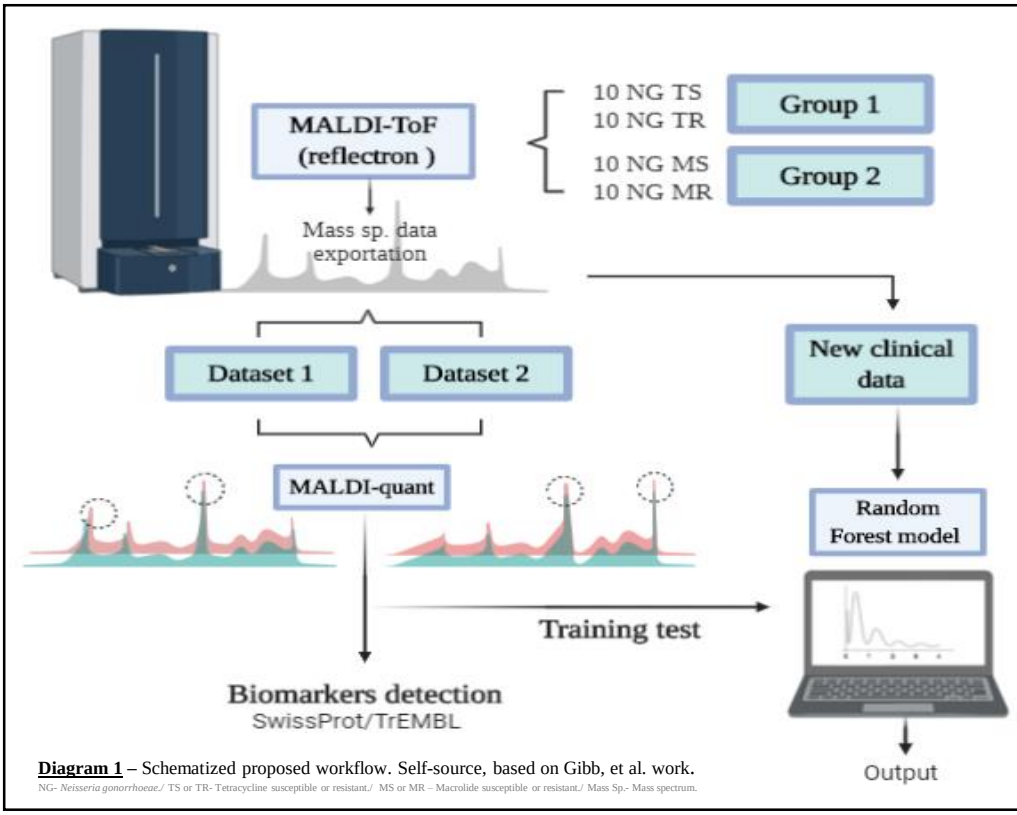

Diagram 1 – Schematized proposed workflow. Self-source, based on Gibb, et al. work.
NG- *Neisseria gonorrhoeae*./ TS or TR- Tetracycline susceptible or resistant./ MS or MR – Macrolide susceptible or resistant./ Mass Sp.- Mass spectrum.

CLINICAL IMPLEMENTATION OF THE PROPOSED WORK: The proof of concept is expected to: – Improve the gonococcal infection treatment by the generation of a more efficient protocol applicable to routine laboratory (decrease in health care expenditure). – Check the MALDI-ToF technique efficiency against phenotyping RB resistances. – Generate a suitable dataset for the development of further molecular and epidemiological studies.

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4. Direct Bacterial Profiling by Matrix-Assisted Laser Desorption–Ionization Time-of-Flight Mass Spectrometry for Identification of Pathogenic Neisseria. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2607569/.