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# Quantitative metabolomics of urine for rapid etiological diagnosis of urinary tract infection: Evaluation of a microbial–mammalian co-metabolite as a diagnostic biomarker

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ABSTRACT

*Background:* We have previously reported a NMR-based urinalysis for the screening of urinary tract infection (UTI) with high accuracy and reproducibility. Urinary acetic acid per creatinine was found to be a diagnostic marker of bacterial UTI with an area-under-receiver operating characteristic (ROC) curve of 0.97. In addition, we identified trimethylamine (TMA) as a human-microbial marker of *Escherichia coli* (EC)-associated UTI. Here, we evaluate the clinical application of NMR-based urinalysis in aiding the etiological diagnosis of bacterial UTI.

*Methods:* Proton NMR spectroscopy was acquired using a Bruker 600 MHz spectroscopy for 88 urine samples from patients with bacterial UTI, confirmed by urine culture. The spectra were analyzed using orthogonal partial least squares-discriminant analysis (OPLS-DA). ROC curve analysis was performed after the quantitation of the urine metabolites.

*Results:* The TMA/creatinine (mmol/mmol) level was determined to be a specific marker for EC-associated UTI. It has an area-under-ROC = 0.85 (95% confidence interval: 0.75–0.91). For the etiological diagnosis, the cutoff for 97.0% specificity was at 0.0117 mmol/mmol creatinine for EC-associated UTI with a sensitivity of 66.7%. The mean of TMA/creatinine of EC is 21-fold that of non-EC.

*Conclusions:* The co-metabolism of TMA by EC and human cells makes TMA an ideal urine biomarker for UTI. The presence of TMA in a freshly collected sample eliminates the possibility of contamination of urine by bacteria during the collection process resulting in a positive bacterial culture result. We envisage the NMR-based urinalysis of urinary TMA that can be a useful method for the etiological diagnosis of EC-associated UTI.



## 1. Introduction

We previously reported a NMR-based urinalysis for the screening of urinary tract infection (UTI) with high accuracy and reproducibility. Using chemometric analysis, we identified that urine acetic acid per creatinine was the most diagnostic marker for UTI with an area-under-receiver operating characteristic (ROC) curve of 0.97, a sensitivity of 91% and a specificity of 95%. The diagnostic performance of NMR-based urinalysis was far superior to dipstick urinalysis or microscopy [1]. This could result in submitting less false positive urine samples for bacterial culture [2].

In this study, we further describe the application of NMR-based urinalysis for the etiological diagnosis of bacterial UTI using quantitative metabolomics. Currently, there is no simple method for the etiological classification of bacterial UTI. Urine culture is considered to be the method of choice and is routinely used in most clinical laboratories [3,4]. It provides useful clinical information such as bacterial load (or colony forming units (CFUs)) and antibiotic sensitivity. However, this method is labor intensive and with a relatively long turnaround time [5,6].

Clearly, there is a need to develop a simpler and faster tool to predict the causative organisms for bacterial UTI. Gupta et al. had shown that 6-hydroxynicotinic acid (6-OHNA) and 1,3-propanediol (1,3-PD) were bacterial specific metabolites of *Pseudomonas* and *Klebsiella* respectively [7,8]. By measuring the 6-OHNA and 1,3-propanediol using $^{1}$H NMR spectroscopy, the *Pseudomonas* associated UTI and *Klebsiella* associated UTI can be determined objectively. This novel NMR-based method had

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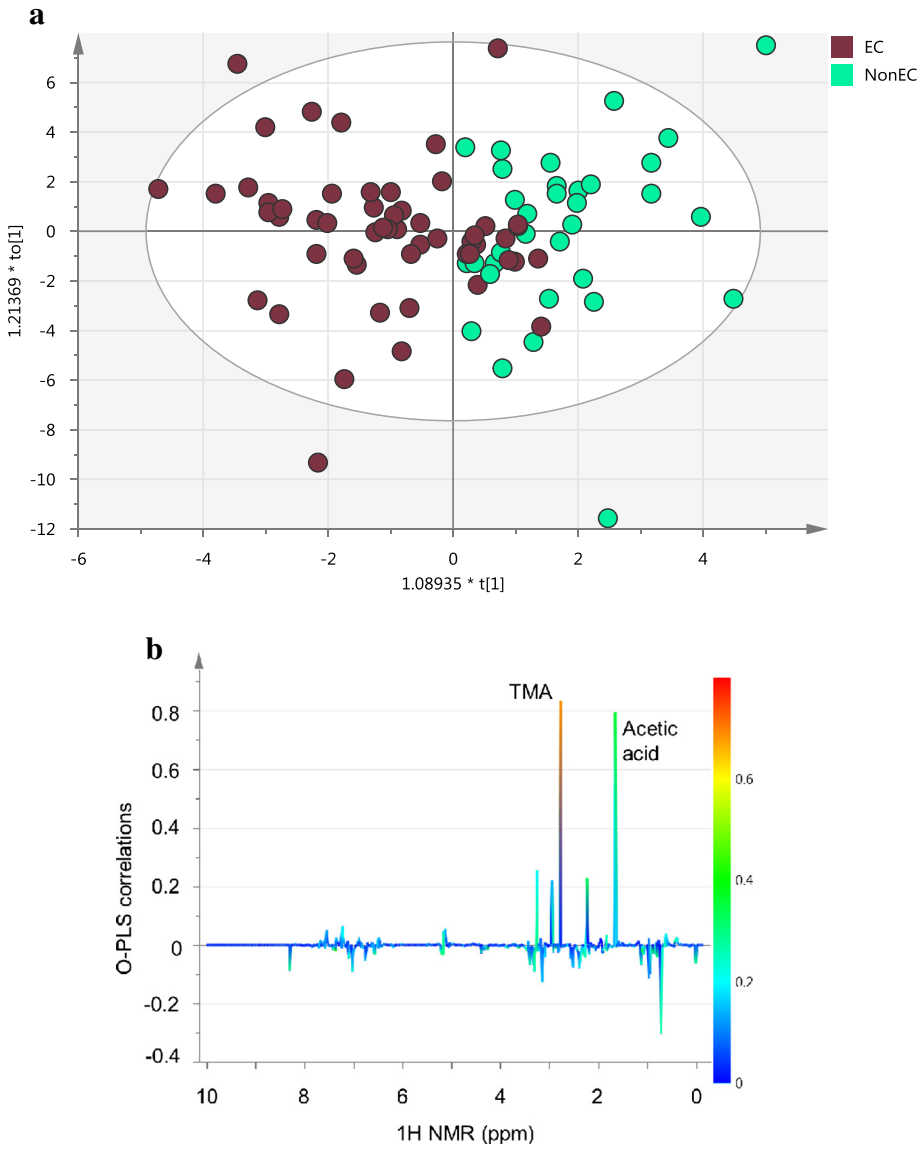


**Fig. 1.** OPLS-DA score plot comparing EC and non-EC associated UTIs (a). OPLS-DA correlation plot comparing EC and non-EC associated UTIs (b).

dramatically improved the turnaround time from days into hours. However, it is still considered to be a labor intensive method since it requires additional sample preparation steps by adding nicotinic acid (precursor of 6-OHNA for *Pseudomonas*) and glycerol (precursor of 1,3-PD for *Klebsiella*) into the urine samples as well as an incubation step.

In this study, we describe a simple and direct approach for the etiological diagnosis of bacterial UTI, in particular EC-associated UTI, the most common cause for all UTI [9]. We will compare the $^{1}$H NMR spectra of EC and non-EC associated bacterial UTIs using chemometric analysis and quantitative metabolomics [10,11]. TMA, a microbial-mammalian co-metabolite was identified to be the most specific marker for EC. The quantitation of urine TMA will be measured by $^{1}$H NMR spectroscopy and the optimal cutoff for clinical use will be determined using ROC curve analysis.

## 2. Material and methods

### 2.1. Sample preparation and investigation

Midstream urine samples were collected in sterile plain bottles. Urine culture was performed according to the standard procedure [12,13], and positive bacterial culture was defined as a bacteria growth $>10^5$ colony forming units (CFUs) per milliliter. An aliquot of the urine sample was used for the NMR experiment. All NMR spectra were acquired on a Bruker Avance 600 MHz NMR spectrometer. The sample preparation and parameters for NMR experiments had been described previously in [1]. The collection of urine samples had been approved by the Institutional Review Board (IRB).

### 2.2. Data processing and statistical analysis

Chemometric analysis, *i.e.* orthogonal partial least squares-discriminant analysis (OPLS-DA), was performed using SIMCA-P13 software (Umetrics) as described in [1].

### 2.3. Quantitative metabolomics

Quantitation of urine metabolites was performed using Chenomx NMR suite 7.0. Details of the chemometric analysis and the quantitative metabolomics had been described in [1]. Other statistical analyses were performed using MedCalc (ver 13.1.0).