1H-NMR Spectroscopy in the Diagnosis of Proteus mirabilis-induced urinary tract infection

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SYNOPSIS: ^{1}H NMR spectroscopic method is suggested and applied for the diagnosis of *Proteus mirabilis (P. mirabilis)* in urinary tract infection (UTI). Specific property of *P. mirabilis* of metabolizing methionine to 4-methyl-2-oxobutyric acid in urine is exploited. Other common bacteria, such as *E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter aerogenes, Acinetobacter baumanii, Citrobacter frundii* do not produce 4-methyl-2-oxobutyric acid. The method was applied to *P. mirabilis* (n=10) infected urine samples and the results showed very high sensitivity with reference to conventional method. Diagnosis of *P. mirabilis* bacteria causing UTI infection in a single step both qualitatively and quantitatively may have useful implications.

INTRODUCTION: In earlier reports we have demonstrated the use of NMR spectroscopy for the bacterial identification and quantification in Urinary Tract Infection (UTI) for *E. coli, K. pneumoniae* and *P. aeruginosa*[1, 2, 3]. Though the above bacteria play major role in the UTI, the *P. mirabilis* also contribute to about 1-3% of the UTI infections[4]. We report herein the application of NMR for the identification of the bacteria, *P. mirabilis* by using its specific property of metabolizing Methionine to 4-methyl-2-oxobutyric acid. Over 617 patients affected by UTI were studied. We found only 240 *E. coli*, 101 *K. pneumoniae*, 56 *P. aeruginosa*, 8 *Proteus mirabilis* by NMR spectroscopic method against 256 *E. coli*, 110 *K. pneumoniae*, 60 *P. aeruginosa*, 10 *Proteus mirabilis* as observed by gold standard culture method and 83 samples were infected by others bacterial infections. In the remaining cases no bacterial metabolites were identified and from the microbiological culture method and they were found to be sterile.

MATERIALS AND METHODS: Standard bacterial strains *E. coli* ATCC-25923; *P. aeruginosa* ATCC-25922; *K. pneumonia* ATCC-13883; *Enterobacter* ATCC-13048*, Acinetobacter* ATCC-19606*, Proteus mirabilis* ATCC-49565*, Citrobacter* ATCC-8090 were used for *in vitro* study to test methionine metabolism using NMR. Each bacterial strain (10^7 cfu/ml) was taken in one ml of sterile urine and treated with the 2 mg of methionine, incubated for 6 hrs at 37^o C and the supernatant solutions were subjected to ^{1}H NMR experiments. Urine solutions for *P. mirabilis* were made with variable bacterial count (10^3, 10^4, 10^5, 10^6 and 10^7) and ^{1}H-NMR spectra were recorded for the supernatant medium obtained after incubation. Urine specimens from 10 patients of urinary tract infection with *P. mirabilis* (about 2 ml each) were obtained from microbiology departments of the medical centers. The bacterial infection was confirmed using conventional culture method. All the 10 urine specimens (1 ml each) were treated separately with 2 mg of methionine, incubated for 6 hrs at 37^o C, and the supernatant solutions were subjected to ^{1}H-NMR experiments. ^{1}H NMR experiments were performed on a Bruker Avance 400 MHz spectrometer. In each case, 600 µl of solution were taken in 5 mm tube and a reusable sealed capillary containing pre-calibrated 25 µl of 0.75% trimethyl silyl propionic acid sodium salt (TSP) deuterated at CH_2 groups dissolved in deuterium oxide was inserted into the NMR tube. Typical parameters used were, spectral width: 8000 Hz; data points: 32K; flip angle: 45^o; number of scans 64; relaxation delay 5s and FT size: 32 K.

RESULTS: ^{1}H NMR spectra indicated that, of all the bacteria, only *P. mirabilis* metabolized methionine to produce 4-methyl-2-oxobutyric acid (Fig. 1). Further, the quantity of 4-methyl-2-oxobutyric acid produced positively correlated with the number of bacteria. Of the 10 urine specimens diagnosed for *P. mirabilis* using the conventional culture method, 8 showed positive test from ^{1}H-NMR.

DISCUSSION: In this study we have shown that among the UTI causing bacteria, only *P. mirabilis* metabolizes methionine into 4-methyl-2-oxobutyric acid as the unique metabolite. This was achieved by growing the standard bacterial strains in urine medium in presence of methionine. Positive correlation of the 4-methyl-2-oxobutyric acid with the bacterial counts indicates that using the methionine metabolism, *P. mirabilis* bacteria can be detected qualitatively as well as quantitatively in a single experiment. Among 10 urine samples diagnosed to be infected with *P. mirabilis*, 8 were having significant number of bacteria (>10^5). NMR method showed positive results for all the 8 urine diagnosed significant using culture method. However, among the 2 urine specimen with insignificant bacteria, NMR showed negative. Since NMR method under the present conditions could not detect bacteria less than 10^3, negative result for 2 urine specimens indicate that the number of bacteria in these specimens is less than 10^3. The fact that both qualitative and quantitative identification of *P. mirabilis*, is possible in one step using NMR indicates the possible utility of NMR based metabonomics in the diagnosis of bacterial infections. These results clubbed with our recent studies[1,2,3] on other three major bacteria of UTI indicate the possibility of rapid and specific identification of the microorganism (*E. coli, P. aeruginosa, K. pneumoniae* and *P. mirabilis*) causing UTI.

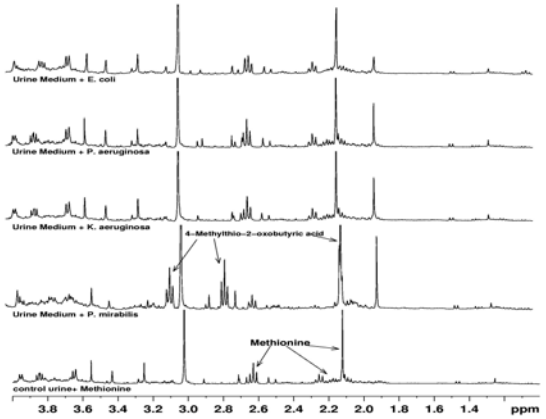

Fig. 1: Major bacteria incubated in urine in presence of methionine. Only *P. mirabilis* produces 4-methyl-2-oxobutyric acid

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