Diagnosis of Pseudomonas aeruginosa and Klebsiella pneumoniae induced urinary tract infection by NMR

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Synopsis: The use of proton NMR spectroscopy for diagnosis of Urinary Tract Infection (UTI) caused by *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Klebsiella pneumoniae (K. pneumoniae)* is proposed. *P. aeruginosa* metabolizes Nicotinic Acid (NA) into 6-Hydroxy Nicotinic Acid (6-OHNA) while *K. pneumoniae* metabolizes glycerol and fumaric acid into 1,3-propanediol, succinate, acetate and ethanol . In suspected UTI patients (n = 200), urinary excretion of *P.aeruginosa* and *K. pneumoniae* were identified by NMR through signals of the respective metabolic products. NMR method showed 53 infected with *P. aeruginosa* and *29* with *K. pneumoniae* versus 55 and 30, respectively, from the conventional culture method.

Introduction: *Escherichia coli* (*E. coli), K. pneumoniae* and *P. aeruginosa* are the main causes of UTI. Conventional culture method of microbial identification is time consuming and labor intensive. Consequently, several new approaches such as Fourier transform infrared spectroscopy, Raman spectroscopy, pyrolysis mass spectrometry, gas liquid chromatography and reagent dipstick method have been reported with an aim of developing techniques for rapid microbial identification. We present herein ^{1}H NMR spectroscopic approach for the identification (both qualitatively and quantitatively) of the bacteria of UTI, uniquely.

Material and Methods: Standard bacterial strains used for establishing Nicotinic Acid (NA) metabolism by *P. aeruginosa* and glycerol & fumaric acid metabolism by *K. pneumonia* were *P. aeruginosa* NCTC-10662, ATCC-25922; *E. coli* NCTC-10418, ATCC-25923; *K. pneumonia* NCTC-9633, ATCC-13883; *Enterobacter* ATCC-13048, *Acinetobacter* ATCC-19606, *Proteus mirabilis* ATCC-49565, *Citrobacter* ATCC-8090, *Enterococcus faecalis* ATCC-19433, *Streptococcus gp B* ATCC-13813, *and Staphylococcus aureus* ATCC 12600. Each bacterial strain (10^7 cfu/ml) was taken in one ml of sterile urine and treated with the substrates (1 mg of NA, 2μl of glycerol and 1 mg of fumaric acid), incubated for 6 hrs and the supernatant solutions were subjected to ^{1}H NMR investigations. All the ^{1}H NMR experiments were performed on a Bruker Avance 400 MHz spectrometer, 600 μl of solution was taken in 5 mm tube and a reusable capillary containing 30 μl of 0.375 % sodium salt of Trimethyl Silyl Propionic acid (TSP) in deuterium oxide was inserted in the NMR tube before obtaining the spectra. Parameters used were, spectral width: 8000 Hz; data points: 32K; flip angle: 45°; number scans 24; relaxation delay 5s and FT size: 32 K. For *P. aeruginosa* and *K. pneumonia,* different numbers of bacteria varying from 10^3 to 10^7 cfu/ml were incubated in presence of the substrates and subjected to NMR analyses as explained above.

Urine specimens were collected from 200 patients of suspected UTI and each sample divided into two parts. One part was used for bacterial identification using conventional culture method. The other part was subjected to NMR analysis; treated with the substrates and incubated for 6 hrs. Subsequently, the supernatant solutions were subjected to NMR experiments. Using the NMR signals, the metabolic products of NA, glycerol and fumaric acid, wherever observed, were quantified with reference to the signal of the TSP. Quantities of *P. aeruginosa or K. pneumonia* in the patients' urine specimen were determined by comparing the concentrations of the metabolic products with those determined from the standard bacteria.

Results: In the NMR spectra of the standard bacterial strains treated with the substrates, signals of 6-OHNA, the metabolic product of NA, were observed for both NCTC and ATCC strains of *P. aeruginosa*. Similarly, the signals of 1,3-propanediol, succinate, acetate and ethanol which are the metabolic products of glycerol and fumaric acid, were observed for both the strains of *K. pneumoniae*. The NMR spectra from the supernatants of the remaining bacterial incubated media showed that the substrates were intact. Further, in the case of *P. aeruginosa* and *K. pneumoniae*, the quantity of the metabolic products was proportional to the number of bacteria.

Bacterial culture of the urine specimen indicated that of the 200 specimens, 85 were infected with *E. coli*, 55 with *P. aeruginosa* and 30 with *K. pneumoniae*. The remaining 30 were sterile. From the metabolism of nicotinic acid to 6-OHNA or of glycerol and fumaric acid to 1, 3-propanediol, acetate, succinate and ethanol as observed by ^{1}H NMR spectroscopic method, 53 specimen were identified as infected with *P. aeruginosa* (out of 55 by the conventional method) and 29 as infected with *K. pneumoniae* (out of 30 by the conventional method). The remaining samples were found to be not infected by either by *P. aeruginosa or* by *K. pneumoniae*.

Discussion: Identification of P. *aeruginosa or K. pneumoniae* was first demonstrated using standard bacterial strains of NCTC and ATCC from their selective metabolism of NA or glecerol and fumaric acid respectively. The appearance of the signals of 6-OHNA or 1,3-propanediol, succinate, acetate and ethanol in the ^{1}H NMR spectra of the UTI patients' urine incubated with the substrates indicates the presence of *P. aeruginosa or K. pneumoniae* respectively. Further, increase in the intensity of the signals of the metabolic products with increase in number of bacteria directly provides quantitative estimation of the bacteria. Bacterial identification from NMR method shows very good specificity (97 %) relative to the conventional culture method.

Preliminary NMR experiments on the standard strains of *E. coli* show that this bacterium metabolizes lactose, selectively producing lactate as one of the metabolites. This would enable the diagnosis of *E.coli* also resulting in enhancing the scope to specifically diagnose the three major bacteria causing UTI.

The method provides a rapid and specific identification of the common bacteria causing UTI.