Diagnosis of Escherichia coli induced urinary tract infection by NMR

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SYNOPSIS: ^{1}H NMR spectroscopic method is suggested and applied for the diagnosis of *Escherichia coli (E. coli)* in the urinary tract infection (UTI). Specific property of *E. coli* of metabolizing lactose to lactate in urine is exploited. Other common bacteria, such as *Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter aerogenes, Acinetobacter baumanii, Proteus mirabilis, Citrobacter frundii* do not produce lactate. The method was applied to *E. coli* (n=96) infected urine samples and the results showed very high sensitivity with reference to conventional method. Diagnosis of *E. coli* bacteria causing UTI infection in a single step both qualitatively and quantitatively may have useful implications.

INTRODUCTION: Among several bacteria, *E. coli, Klebsiella pneumoniae (K. pneumoniae)* and *Pseudomonas aeruginosa (P. aeruginosa)* are the main bacteria causing UTI. Conventional culture method of microbial identification is time consuming and labor intensive. There have been several new approaches for rapid diagnosis of bacterial infections. Efforts are continuing to develop simple test for rapidly identifying bacteria both qualitatively and quantitatively. Recently, we have presented ^{1}H NMR spectroscopy method to diagnose *P. aeruginosa* and K. *pneumoniae* from the urine of UTI patients.[1,2] In continuation, in this study we have explored application of NMR spectroscopy to diagnose *E. coli* infections of urinary tract. The method of detection of *E. coli* in urine which is essentially based on the bacteria metabolizing lactose to lactate is tested on urine specimens from 96 UTI patients.

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 lactose 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 lactose, incubated for 6 hrs at 37° C and the supernatant solutions were subjected to ^{1}H NMR experiments. Urine solutions for *E. coli* 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 for 6 hrs at 37°C. Urine specimens from 96 patients of urinary tract infection with *E. coli* (about 2 ml each) were obtained from microbiology departments of the medical centers. The bacterial infection was confirmed using conventional culture method. All the 96 urine specimens (1 ml each) were treated separately with 2 mg of lactose, incubated for 6 hrs at 37° 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 was taken in 5 mm tube containing a reusable co-axial capillary having calibrated quantity (140 μg) of reference compound, sodium salt of trimethylsilylpropionic acid (TSP) in 35 μl of deuterium oxide. Typical parameters used were, spectral width: 8000 Hz; data points: 32K; flip angle: 45°; number scans 64; relaxation delay 5s and FT size: 32 K. Wherever lactate signal was observed in the spectra, its concentration was determined from the integral area of the methyl signal at 1.32 ppm relative to reference, TSP.

RESULTS: ^{1}H NMR spectra indicated that, among all the bacteria, only *E.coli* metabolized lactose to produce lactate (Fig. 1a). Further, the quantity of lactate produced positively correlated with the number of bacteria (Fig. 1b). Among the 96 urine specimens diagnosed for E. coli using the conventional culture method, 91 showed positive test from ^{1}H NMR (Fig. 1c).
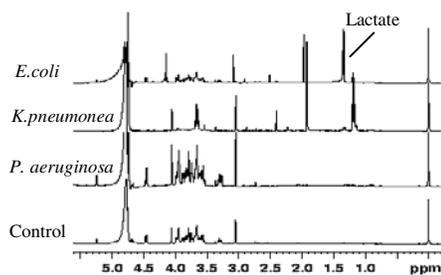

Fig. 1a: Major bacteria incubated in urine in presence of lactose. Only *E. coli* produces lactate
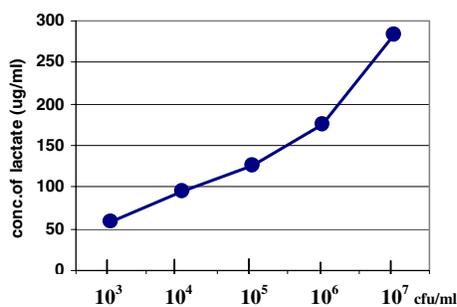
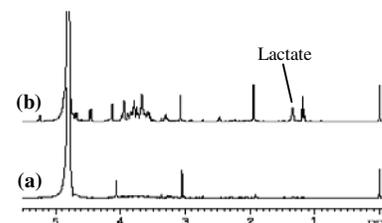**Fig. 1b:** Concentration of lactate Vs bacterial colony count.

Fig. 1c: Typical urine from E. coli infected urinary tract (a) without lactose (b) with lactose. Lactate signal confirms *E. coli* infection

DISCUSSION: In this study we have shown that among the major bacteria causing UTI, only *E. coli* metabolizes lactose into lactate as the unique metabolic end product. This was achieved by growing the standard bacterial strains in urine medium in presence of lactose. Positive correlation of the lactate with the bacterial count as seen in Fig. 1b indicates that using the lactose metabolism, *E. coli* bacteria can be detected qualitatively as well as quantitatively in a single experiment. Among 96 urine samples diagnosed to be infected with E.coli, 65 were having significant number of bacteria ($>10^5$) and 31 were insignificant ($<10^5$). NMR method showed positive results for all the 65 urine diagnosed significant using culture method. However, among the 31 urine specimen with insignificant bacteria, NMR showed positive for only 26. Since NMR method under the present conditions could not detect bacteria less than 10^3, negative result for 5 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 *E. coli*, the most abundant bacteria of UTI, 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] on other two major bacteria of UTI indicate the possibility of rapid and specific identification of the major three bacteria (*E. coli, P. aeruginosa, and K. pneumoniae*) causing UTI. Our ongoing studies to identify these three major bacteria in urine samples in a single step using NMR show promising results.

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