INTRODUCTION

Urinary tract infections (UTI) pose serious health problems throughout the world affecting millions of people each year. UTIs rank second only to respiratory infections in the United States and account for about 9.6 million doctor visits annually. However, many of the urine samples that are received by the clinical laboratories may contain contaminants only and very few or no infecting microbes. The current techniques that are used for the detection and identification of microorganisms include microscopy, growing different cultures, and more recently DNA based methods such as PCR. Though these methods are the most preferred ones, their main disadvantage is the slow speed of analysis.

In contrast, selected ion flow tube mass spectrometry (SIFT-MS) simultaneously detects and quantifies volatile organic compounds (VOCs) in real time that are produced by the infecting organisms to pptv levels with no sample preparation. In this way, if different organisms produce unique types of compounds then monitoring those compounds aids the detection and identification of the infection.

We show here how readily a SIFT-MS instrument can be applied to measure the microbial VOCs (MVOCs) occurring in the headspace of urine samples inoculated with UTI-causing microbes such as E. coli, P. vulgaris, P. aeruginosa, S. aureus, S. epidermidis, C. albicans etc.

METHOD

SIFT-MS uses ultra-soft, precisely controlled chemical ionization coupled with mass spectrometric detection (Figure 1) to rapidly detect and quantify VOCs and inorganic gases to low part-per-trillion concentrations by volume (pptv). Eight different microbial species were used in the study (10 replicate samples of each). The samples were prepared using thawed sterile urine (20 mL) from healthy males inoculated to a concentration of between 107 and 109 cfu/mL and incubated at 37°C for 6 h. The headspace of each urine sample was then tested through the sample inlet of a Voice200. Each sample was assayed using SIFT-MS in SIM scan mode for 45 seconds.

Each VOC was monitored using product ion masses that were selected to avoid masses that would overlap with other known compounds. Because the reaction rates between the analytes and the reagent ions are known, quantification is achieved in near-real time using on-board software and the in-built library.

RESULTS AND DISCUSSION

The approximate microbe concentration was determined by comparing the standard bacterial growth curves (optical density measurements at 580 nm and 686 nm). Spread plates prepared from the inoculated urine showed very heavy but homogenous growth after 24 hour incubation period indicating inoculation of single bacterial strain. The blank urine control plates showed no growth after 24 hour incubation indicating no contamination from external sources.

Figure 2 shows the sample MVOC mean concentrations minus the mean blank/negative controls + 2 standard deviations detected using SIFT-MS technique. Negative values show the microorganism has used the compound. These data illustrate (i) the simplicity with which SIFT-MS can be applied to profiling chemically diverse volatile compounds in a single analysis, and (ii) the differentiation of different strains of microorganisms, which enables detection and identification of the microorganisms associated with different types of UTIs.

Despite having differences in culture media and sampling procedures there was some agreement in the current finding and previous studies. For example, S. aureus produced significant amounts of ammonia and dimethyl disulfide, P. vulgaris produced methyl mercaptan, production of indole indicated E. coli. However, there was no significant increase in 2-aminoacetoephone which was found to indicate the presence of P. aeruginosa. A possible reason is that earlier studies used different culture media and a different analysis technique.

CONCLUSIONS

The real-time, comprehensive analysis provided by SIFT-MS makes it ideally suited for the rapid detection and quantitation of volatile metabolites which aids in the early diagnosis of Urinary Tract Infections. Also, having a unique set of volatile metabolites for each microorganism further aids the detection and identification of microorganisms associated with different types of UTIs. Early identification also helps in providing the right treatment to the patient.

REFERENCES