ABSTRACT

Control of infectious diseases through early identification of pathogens, or better still, surveillance to eradicate is becoming more and more meaningful with the emergence of Multi-drug-resistance (MDR) and spread of dangerous pathogenic forms from hospitals to communities. The most common and prevalent Urinary Tract Infections (UTI) are also one of the most neglected infectious diseases. The classical and current techniques for diagnosis are not effective for a variety of reasons including the nature of the diagnostic targets and methods. Hence, its treatment is quite challenging making it imperative to develop quick diagnosis and render antibiotic treatment effective. Taking one of the notorious nosocomial causative bacterium, *Proteus*, we have addressed the challenge making a paradigm shift in the approach of detecting the bacteria.

In this regard, Volatile Organic Compounds (VOCs) which are secreted as defense against antagonists or as signalling molecules by the organisms under specific conditions through specific biochemical pathways were exploited. In the case of *Proteus*, 2-methylbutanal identified by GC-MS was found to be the characteristic volatile compound released in Luria Bertani (LB) broth. Using this compound we were able to develop a simple test in 96-well microplate format that can be directly applied to the 7 h culture of the bacterium to give a yes-or-no type of response for fluorimetric detection. The assay, named *ProteAl*, (*Prote*, “*Proteus*” & Al, “Aldehyde”) involves instant reaction of 5-dimethylaminonaphthalene-1-sulfonylhydrazine (DNSH) with
2-methylbutanal under acidic condition to give green fluorescence (other organisms show orange fluorescence).

This diagnostic assay has been tested using 39 standard and 56 known clinical strains representing frequently encountered uropathogens including \{27 Proteus (both mirabilis and vulgaris), 27 E.coli, 8 Klebsiella, 10 Staphylococcus, 7 Pseudomonas\}, 2 Enterobacter, 2 Citrobacter, 7 Salmonella, 4 Shigella and 200 environmental soil strains. The sensitivity and specificity of this high-throughput assay performed in 96-well format were 100% under laboratory conditions and therefore forms the basis for larger clinical validation. This cost-effective diagnostic tool will be useful in hospitals, peripheral clinics, epidemiological studies and environmental surveillance.

Metabolic pathway and regulation studies (including qPCR) based on the limited reports available in a few other systems revealed the presence of functional pathway in Proteus and its regulation through Isoleucine (Ile) and Thiamine pyrophosphate (TPP). This led to the designing of LB-Ile medium with 15 mM isoleucine in LB to enhance the production of the biomarker 2.5 times more than normal. The growth in the rationally designed medium and ProteAl now would provide a convenient diagnostic tool for identifying this bacterium from clinical samples within 7 h. The expression of alpha-ketoacid decarboxylase (kdcA) of Proteus grown in LB-Ile medium revealed a seven-fold increase in expression compared to normal LB. This indicated to the operation of transcriptional control in Proteus and this is the first such report revealing the existence of isoleucine catabolism in Proteus (mirabilis and vulgaris).
Though we have focused on *Proteus* associated with UTI, the method is genus specific and therefore can be used for other disease conditions. The development of such cost effective, non-invasive and non-destructive method has been shown to be readily amenable for simple imaging based instrumentation (like gel doc) for routine clinical use. In conclusion, we have taken a new approach towards next generation diagnostic method for infectious bacteria that can be readily adapted to instrumentation and automation.