

Introduction

Clostridium difficile infection produces a range of effects from asymptomatic carriage to severe diarrhoea, colitis and death.

While usually considered a nosocomial infection consequent on exposure to antibiotics, transmission in the community is becoming increasingly recognised while another emerging issue is the consequences of infection in vulnerable groups, notably children and those suffering from inflammatory bowel disease. The increased incidence of *Clostridium difficile* infection is associated with the emergence of strains with enhanced virulence resulting from increased toxin production and antibiotic resistance.

Early diagnosis of *Clostridium difficile* infection is therefore essential for targeted therapy, the prevention of spread and effective use of resources.

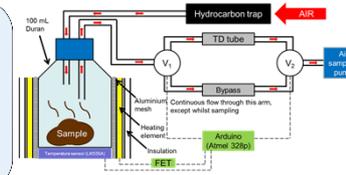


Figure 1 Headspace sampling system

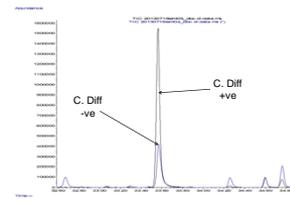


Figure 2 Portions of representative chromatograms of *C. diff* positive and negative samples. The principle peak visible represents 4-methyl-phenol (*para*-cresol).

Volatile organic compounds and bacteria

It has been established since the 1980s that bacteria grown in culture can be identified on the basis of the different mixtures of volatile organic compounds (VOCs) which they emit into the surrounding air. Moreover, substantial anecdotal evidence points to a distinctive odour associated with the faeces of patients suffering from *Clostridium*-associated disease. We hypothesised that this odour was associated with the presence of the active bacterium and that the volatile organic compounds giving rise to it would be detectable analytically.

Based on a literature search, a group of thirty VOCs was selected for investigation.

Methods

Stored, frozen faecal samples were obtained from the Centre for Infection Control at Guy's and St. Thomas Hospital, London. All been characterised as *C. diff* positive or negative using a combination of enzymatic and molecular tests. Samples (53 each of positive and negative) were placed in a novel sample handling system (Figure 1) and headspace extracted onto thermal desorption tubes for subsequent analysis by thermal desorption – gas chromatography – mass spectrometry (TD-GC-MS).

This system maintains the sample at a constant temperature under a constant flow of clean air. After an equilibration period, the flow is diverted through a thermal desorption (TD) tube which captures the emitted VOCs. This approach allows the rate of emission of individual VOCs to be determined.

Results

Headspace concentrations of each compound were quantified and receiver-operator characteristic (ROC) curves for classification of the samples constructed. Eight compounds emerged as potential biomarkers: 1-propanol, 3-methyl-butanol, propanoic acid ethyl ester, isovaleric acid, 4-heptanone, 4-methyl-phenol, indole and hexanoic acid. The first seven of these were significantly more abundant in positive than in negative samples, while hexanoic acid was found to be an absence marker. Areas under the ROC curves ranged from 0.70 to 0.85 suggesting a diagnostic performance similar to that of current enzymatic methods.

Of these compounds 4-methyl-phenol (*para*-cresol) is of particular interest since it is produced exclusively by *Clostridium* species and exerts a bacteriostatic action on other species. Figures 3 and 4 illustrate the ability of 4-methyl-phenol to discriminate *C. diff* positive and negative faecal samples. Area under the ROC curve was 0.82 (with lower and upper 95% CIs of 0.73 and 0.90).

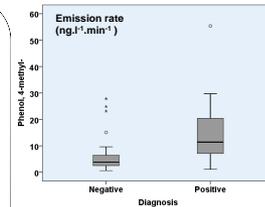


Figure 3 Box plot of emission rate of 4-methyl-phenol from faecal samples diagnosed as positive or negative by conventional tests.

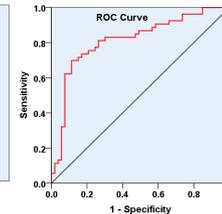


Figure 4 Receiver-operator characteristic curve for 4-methyl-phenol emission rate as a classifier of *C. diff* positive and negative faecal samples.

Conclusions

We have developed a headspace sampling system capable of capturing VOCs emitted by faecal samples and which allows the emission rate of VOCs to be determined.

A number of VOCs were identified which discriminated well between samples taken from patients diagnosed as either negative or positive for *Clostridium difficile* by standard clinical laboratory testing.

One compound, 4-methyl-phenol (*para*-cresol), is of particular interest for future development.

Faecal VOC analysis has potential as a novel diagnostic modality in gastrointestinal infection.

