Rapid identification of bacteria in blood cultures by mass-spectrometric analysis of volatiles

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LETTER TO THE EDITOR

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INTRODUCTION

Blood cultures are routine tests to determine whether micro-organisms have entered the patient's bloodstream. Automated systems, based on the detection of CO_2 increase in the culture media, have considerably improved the screening efficiency for the detection of bacteria.[1] However, further identification of bacteria still requires time-consuming culturing procedures.

It has been suggested that along with CO_2, bacterial cultures emit characteristic volatile organic compounds that may be valuable for characterisation.[2] Recently, a number of technological developments have allowed the detection of trace gases with minimal or no sample preconcentration steps.[3–5] In the current study, we investigated whether the volatiles emitted from bacterial cultures (mimicking routine clinical blood cultures) could be distinguished from each other in a rapid fashion by secondary electrospray ionisation-mass spectrometry (SESI-MS).[6 7]

METHODS

10^3 colony forming units were inoculated in 42 aerobic bottles: 15 with *Staphylococcus aureus* ATCC 29213 strain, 15 with *Escherichia coli* ATCC 25922 and 12 *Streptococcus pneumoniae* ATCC 49619. They were incubated in the automatic instrument BacT/ALERT 3D (Biomerieux Clinical Diagnostic, France). The system automatically detects the positivity of the sample when the amount of CO_2 produced by bacterial growth reaches a given threshold. The bacterial identity was confirmed by culturing according to UK guidelines.

The headspace gas of the positive bottles was sampled with a 5 mL syringe. The gas was subsequently injected into a modified high-resolution mass spectrometer (Orbitrap, Thermo) located in the hospital premises. As a result, an immediate mass spectral fingerprint was obtained. The mass spectra were subjected to one-way analysis of variance (ANOVA) test and principal component analysis (PCA). A k-nearest-neighbour (k-nn, k=1) classifier was employed to predict sample class in a leave-one-out cross-validation (LOOCV).

RESULTS

An immediate mass spectral fingerprint was obtained from all samples. Thirty-three signals in the range 57–308 m/z were found to arise above the background upon injection of the gas. Table 1 lists their m/z values, molecular formula, mean (SD) and p value resulting from the ANOVA test. The average intensity of 27 out of the 33 compounds detected was higher in at least one of the bacterial strains than in the medium. Figure 1 shows an example of the real-time measurement of nine samples (three biological replicates of each bacteria strain) for six representative compounds. Note that each measurement requires a few seconds. Thus, in approximately 10 min, rich volatile fingerprints were obtained for the nine samples. Note how the signal rises upon the injection of the sample with a characteristic intensity, depending on the bacterial type, with a reasonable reproducibility for the three different biological replicates. For example, the compound at m/z 119.0887 ($C_6H_{15}S$) was present in *S. aureus* and *E. coli*, but not in *S. pneumonia* (figure 1A) and the medium (table 1).

The corresponding PCA score plot (figure 2) of the mass spectral fingerprints reveals a clear clustering according to bacterial specimen.[8] The LOOCV resulted in 40 out of 42 (95.2%) samples correctly classified. One *S. aureus* and one *S. pneumoniae* sample were misclassified as *E. coli*.

CONCLUSIONS

This exploratory study suggests that it is possible to identify bacteria by analysing the gases produced in the headspace of blood cultures by mass spectrometry in a rapid fashion with no sample preparation. Consistently with recent real-time mass

Table 1 Volatile compounds detected in real-time in the head-space of the three bacteria investigated

m/z	Molecular formula	*Staphylococcus aureus* (n=15) mean (SD)	*Escherichia coli* (n=15) mean (SD)	*Streptococcus pneumonia* (n=12) mean (SD)	Medium (n=15) mean (SD)	p-Value (ANOVA)
57.07028		1.46 (0.31)	1.45 (0.47)	1.89 (1.09)	1.11 (0.63)	1.9E-01
59.04946	C_3H_7O	6.03 (5.48)	6.57 (5.57)	3.41 (2.42)	3.81 (2.55)	2.2E-01
69.06999	C_5H_9	5.53 (1.81)	7.47 (2.62)	8.82 (2.93)	5.48 (2.02)	4.9E-03
71.08584	C_5H_{11}	0.94 (0.2)	2.32 (1.32)	1.19 (0.99)	0.51 (0.25)	7.7E-04
73.04711		*29.08 (15.25)*	*20.82 (15.1)*	*23.55 (12.98)*	*32.27 (10.25)*	*3.0E-01*
73.06511	C_4H_9O	16.05 (6.4)	30.47 (13.77)	30 (13.61)	13.79 (5.49)	2.2E-03
89.04203		*1.9 (1.64)*	*0.87 (0.99)*	*2.01 (1.45)*	*2.98 (2.61)*	*6.2E-02*
89.05991	$C_4H_9O_2$	16.37 (7.53)	29.43 (23.98)	22.06 (6.88)	12.34 (18.6)	8.1E-02
91.05756	*$C_4H_{11}S$*	*57.46 (34.51)*	*40.69 (27.27)*	*32.04 (18.56)*	*67.07 (37.54)*	*6.7E-02*
95.0609		0.37 (0.33)	0.64 (0.32)	0.42 (0.26)	0.25 (0.33)	5.1E-02
95.08561		2.28 (0.96)	2.19 (1.03)	2.68 (0.76)	1.85 (0.74)	3.6E-01
97.06487	C_6H_9O	6.47 (1.39)	6.69 (1.64)	8.37 (3.42)	4.12 (1.46)	7.4E-02
105.0547	$C_4H_9O_3$	4.13 (2.91)	9.17 (5.23)	10.85 (3.01)	0.43 (0.7)	1.5E-04
105.073	*$C_5H_{13}S$*	*61.17 (33.58)*	*28.99 (25.86)*	*63.24 (34.16)*	*73.26 (36.1)*	*8.2E-03*
105.0911	$C_5H_{13}O_2$	0.89 (0.47)	1.42 (0.76)	1.22 (0.69)	0.5 (0.33)	9.3E-02
109.076	$C_6H_9N_2$	2.9 (1.66)	3.86 (1.69)	3.74 (1.24)	0.95 (1.16)	2.1E-01
109.1012		4.41 (2.88)	10.47 (11.34)	11.17 (7.03)	2.53 (1.3)	5.5E-02
113.0961	$C_7H_{13}O$	8.78 (4.45)	16.48 (8.86)	18.26 (5.1)	3.99 (1.3)	9.2E-04
115.0754	$C_6H_{11}O_2$	75.58 (23.7)	77.72 (18.78)	90.1 (13.65)	37.04 (20.75)	1.4E-01
119.0887	$C_6H_{15}S$	46.51 (21.06)	69.7 (32.42)	2.42 (2.92)	2.4 (1.52)	2.4E-08
119.1066		5.35 (2.25)	5.31 (1.72)	6.3 (1.99)	2.57 (1.26)	3.7E-01
127.1118	$C_8H_{15}O$	10.14 (3.71)	19.12 (14.96)	20.14 (7.1)	6.29 (2.21)	2.0E-02
131.0887	$C_7H_{15}S$	13.49 (8.18)	11.5 (7.63)	19.66 (20.32)	16.57 (10.68)	2.5E-01
142.0631		1.73 (2.29)	5.61 (5.31)	5.35 (4.24)	0.1 (0.39)	2.6E-02
145.1043	*$C_8H_{17}S$*	*3.84 (2.79)*	*3.6 (2.44)*	*3.14 (2.5)*	*5.51 (3.78)*	*7.8E-01*
145.1223	$C_8H_{17}O_2$	9.92 (8.96)	28.41 (16.95)	28.85 (10.78)	3.42 (2.98)	2.7E-04
162.1489	$C_8H_{20}NO_2$	3.33 (2.34)	5.87 (4.13)	5.46 (3.86)	2.92 (2.96)	1.2E-01
163.0969	*$C_7H_{15}O_4$*	*25.35 (8.43)*	*17.83 (8.83)*	*13.32 (5.03)*	*29.7 (12.1)*	*9.7E-04*
177.1485	$C_9H_{21}O_3$	1 (1.35)	3.18 (2.29)	3.74 (2.9)	0.18 (0.29)	5.3E-03
203.1641	$C_{11}H_{23}O_3$	0.99 (1.89)	2.3 (2.74)	2.42 (3.32)	0.71 (1.44)	2.9E-01
219.1745		0.51 (0.39)	1.41 (1.06)	1.76 (1.75)	0.31 (0.24)	1.8E-02
233.2112	$C_{13}H_{29}O_3$	2.15 (3.2)	7.36 (6.24)	8.45 (7.07)	0.18 (0.36)	1.1E-02
308.2796	$C_{14}H_{36}O_3N_4$	6.32 (12.01)	36.31 (39.34)	30.44 (41.73)	5.78 (12.84)	4.4E-02

Average signal of compounds in italics was found to be higher than for the three pathogens investigated.

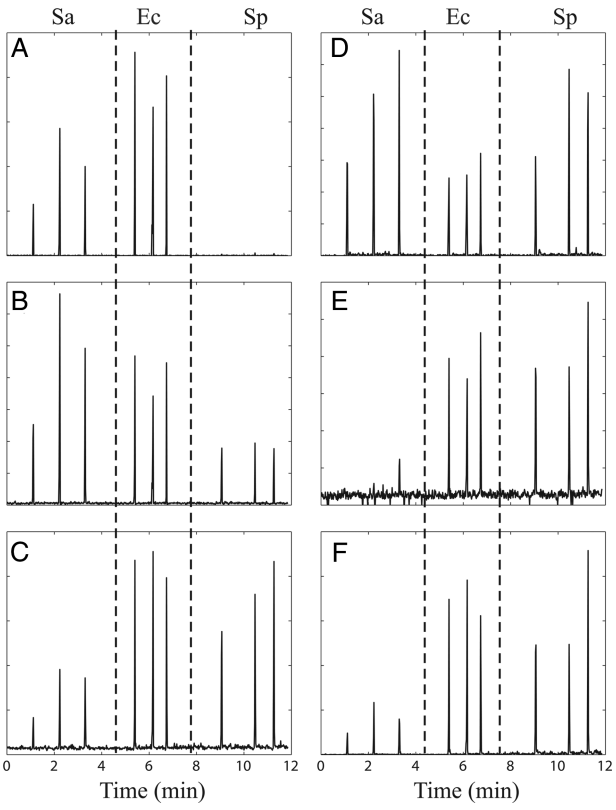

Figure 1 The headspace of commercial blood cultures can be sampled and mass analysed in a few seconds, providing an indication of the pathogen present. The plot displays the intensity as a function of time of six exemplary compounds detected in the headspace of the bacterial cultures. Three biological replicates of *Staphylococcus aureus* (Sa), *Escherichia Coli* (Ec) and *Streptococcus pneumonia* (Sp) were injected at minutes 1.1, 2.2, 3.3; 5.4, 6.1, 6.7 and 9.1, 10.5, 11.3, respectively (A) m/z 119.0887 ($C_6H_{15}S$); (B) m/z 163.0969 ($C_7H_{15}O_4$); (C) m/z 73.06511 (C_4H_9O); (D) m/z 105.073 ($C_5H_{13}S$); (E) m/z 219.1745; (F) m/z 308.2796 ($C_{14}H_{36}O_3N_4$).

spectrometric studies of bacterial cultures,[3 4] relatively low molecular weight volatiles like acetone (m/z 59), acetoin (m/z 89) and butanal (m/z 73) were found in the cultures' headspace. In addition to this, SESI-MS captures rich fingerprints of volatiles expanding well beyond 300 Da.[9] This data suggests that such high molecular weight vapours may be key to deliver high accuracy in pattern recognition of bacteria. For example, the ion detected at m/z 308.2796 ($C_{14}H_{36}O_3N_4$), was found to be the second largest relative contributor in PC3, which essentially separates *E. coli* from *S. aureus* and *S. pneumonia* (see loadings plot in online supplementary figure S1). However, this pilot study has some limitations. For example, during this exploratory phase, we wanted to determine the feasibility of this approach by using pure bacterial standards with relatively high colony-forming units. Further research is needed to confirm these findings in expectedly more complex matrices like blood cultures from patients.

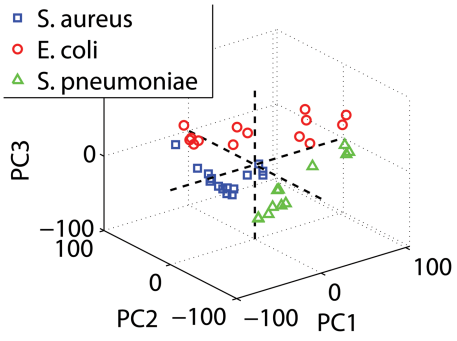

Figure 2 Proof-of-principle of rapid characterisation of three bacterial strains grown in blood bacterial culture vials. Score plot of principal component analysis of the mass spectral fingerprint of the volatiles emitted by three different bacterial agents: *Staphylococcus aureus* (n=15 samples), *Escherichia coli* (n=15) and *Streptococcus pneumonia* (n=12). Clustering according to pathogen is evident, suggesting that before disposal of the positive vials, the analysis of its headspace may be a valuable source of information. Note that each of the measurements shown in the graph requires just a few seconds.

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Contributors SC, PB and PM-LS designed the study. CB, SC, MRS and PM-LS conducted the experiments. PM-LS analysed the data. SC, GP, MK, MRS, PB and PM-LS wrote the manuscript.

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Notes