Supplementary Information

Microbial volatiles as diagnostic biomarkers of bacterial lung infection in mechanically ventilated patients

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Headspace sample collection

An active sampling method was used to sample a total of 200 mL of headspace gas, using a low flow pump (ACTI-VOC sampler, Markes International, Bridgend, UK) at 100 mL min^{-1} onto conditioned tubes containing TenaxGR sorbent material. Headspace gas within the vial was displaced with filtered air which had passed through a secondary TenaxGR sorbent tube. The second method involved passive sampling using polydimethylsiloxane (PDMS) probes (HiSorb^{TM}, Markes International, Bridgend, UK). Bacterial suspensions were cultured and standardised in the same way as for active sampling. Conditioned probes were inserted into the headspace for further 1 h after 23 h of incubation, at 37 °C under shaking conditions. After removal, probes were then dry purged at room temperature for 4 min with a 50 mL min^{-1} flow of N_2 to remove excess water. For both methods, samples without culture (nutrient broth only) were used as negative controls in order to assess background VOCs arising from the sorbent material, glass vials, and analytical process.

Analysis by thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS)

Prior to desorption all samples were spiked with an internal standard (100 µL of 1 ppmv, 4-bromofluorobenzene in N_2; Thames Restek, UK). Tubes went through a two-stage thermal desorption process (TD-100, Markes International, Bridgend, UK) with an initial desorption temperature at 280°C (3 min hold) where VOCs were transferred onto a general purpose hydrophobic focusing trap kept at 0 °C. The trap was then flash desorbed at 280 °C (2 min hold) transferring VOCs into a GC column (DB-5ms 30 m × 0.25 mm x 0.25 µm, Agilent technologies, Cheadle, UK) with a helium carrier gas at constant pressure (69 kPa). The GC oven (7890B, Agilent technologies, Cheadle, UK) was programmed with a ramped temperature gradient starting at 40 °C, ramped to 170 °C at 6 °C min^{-1}, then to 190 °C at 15 °C min^{-1} and finally a 2 min hold at 250 °C (total GC cycle time of 23 min). Breath samples underwent splitless injection, whereas culture headspace samples were split 1:10 to minimise overloading the detector and prevent risk of carry-over. After chromatographic separation, VOCs were

transferred to a mass spectrometer (7010, Agilent technologies, Cheadle, UK) and subjected to electron ionisation (70 eV) in a High Efficiency Source (Agilent technologies, Cheadle, UK). Mass spectra were acquired in full scan mode between *m/z* 40-500 at 5 *Hz*.

Table S1. Intraclass correlation coefficients (ICC) for mVOCs detected in breath using two sequential breath samples from the same patient (n= 83 patient sets available).

Compound	**ICC (95% CI)**
acetone	0.92 (0.88-0.95)
dimethyl sulfide	0.98 (0.97-0.99)
ethyl acetate	0.87 (0.80-0.92)
3-methylbutanal	0.56 (0.33-0.72)
1-butanol, 3-methyl-	0.72 (0.57-0.82)
dimethyl disulfide	0.89 (0.83-0.93)
3-methylbutanoic acid	0.80 (0.70-0.87)
2-heptanone	0.92 (0.88-0.95)
benzaldehyde	0.81 (0.71-0.88)
2-nonanone	0.81 (0.71-0.88)
1-undecene	0.49 (0.21-0.67)
2-phenylethanol	0.64 (0.44-0.76)
indole	0.98 (0.96-0.98)
2-aminoacetophenone	0.89 (0.84-0.93)

ICC = Intraclass correlation coefficient, CI = confidence interval

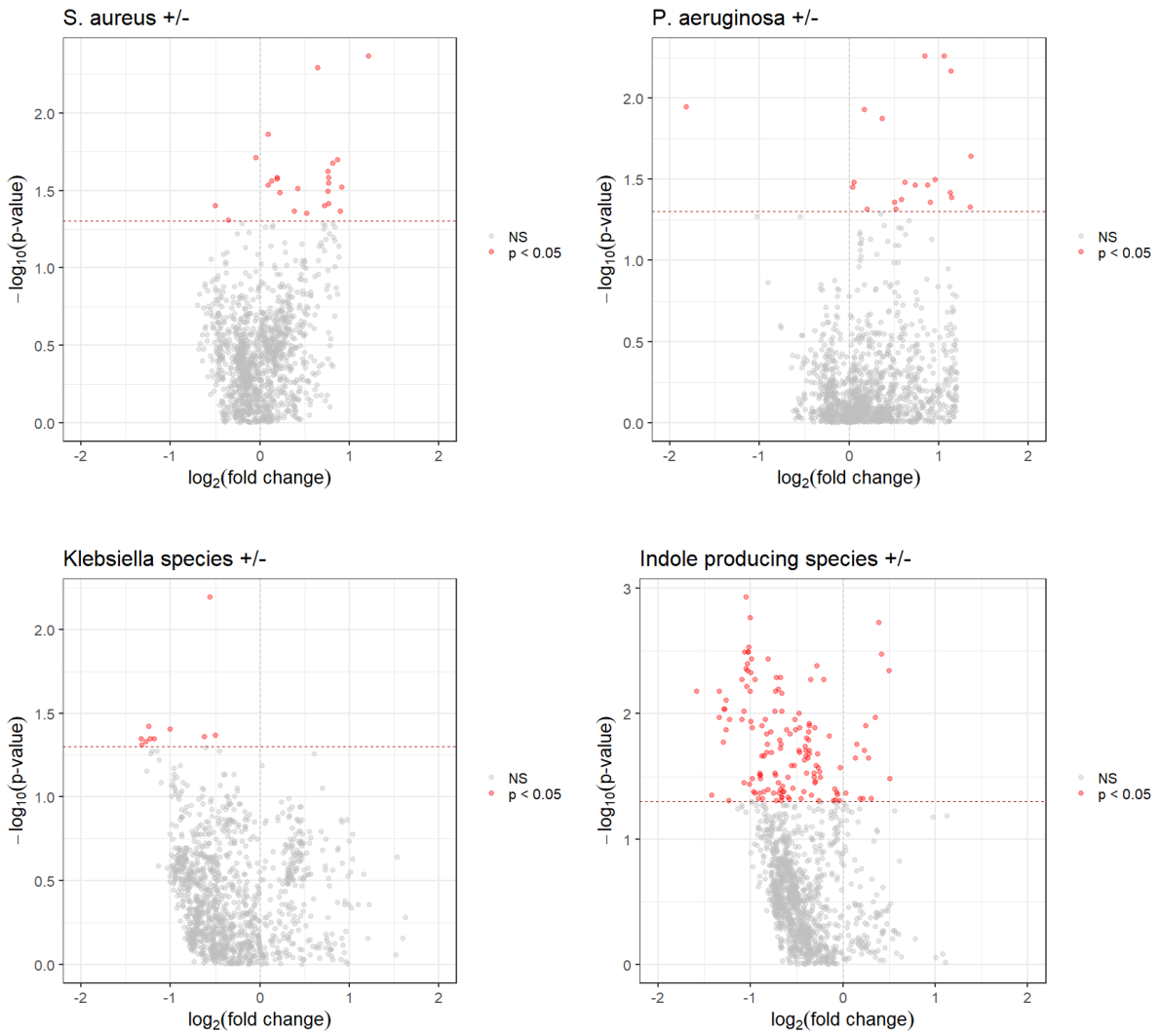

Figure S1. Volcano plots comparing positive and negative culture of pathogens *S. aureus* (top left) and *P. aeruginosa* (top right) and pathogen groups of *Klebsiella* species (bottom left) and indole-producing species (bottom right) using untargeted BreathDx data from van Oort *et al*.[1]. Mann Whitney U test was used to calculate p values.

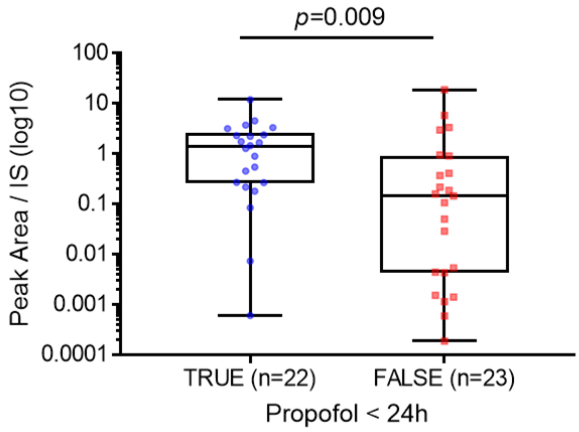

Figure S2. Boxplot comparing propofol in breath samples of patients administered the anaesthetic agent within 24 hours.

References

1. van Oort, P.M., et al., *Untargeted Molecular Analysis of Exhaled Breath as a Diagnostic Test for Ventilator-Associated Lower Respiratory Tract Infections (BreathDx).* Thorax, 2022. **77**(1): p. 79-81.