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Volatile compounds characteristic of sinus-related bacteria and infected sinus mucus: Analysis by solid-phase microextraction and gas chromatography–mass spectrometry

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ABSTRACT

Volatile compounds from human breath are a potential source of information for disease diagnosis. Breath may include volatile organic compounds (VOCs) originating in the nasal sinuses. If the sinuses are infected, disease-specific volatiles may enter exhaled air. Sinus infections are commonly caused by several known bacteria. We examined the volatiles characteristic of infectious bacteria in culture using solid-phase microextraction to collect and gas chromatography–mass spectrometry as well as gas chromatography with flame photometric detection to separate and analyze the resulting VOCs. Infected sinus mucus samples were also collected and their VOCs examined. Similar characteristic volatiles were seen from both cultures of individual "pure" bacteria and several mucus samples. However, the relative amounts of characteristic VOCs from individual bacteria differ greatly between cultures and sinus mucus. New compounds, not seen in culture were also seen in some mucus samples. Our results suggest an important role for growth substrate and environment. Our data further suggests that in some sinus mucus samples identification of bacteria-specific volatiles is possible and can suggest the identity of an infecting organism to physicians. Knowledge of these bacteria-related volatiles is necessary to create electronic nose-based, volatile-specific sensors for non-invasive examination for suspected sinus infection.

1. Introduction

Humans emit a variety of volatile organic compounds (VOCs) from their breath and from discrete body areas [1–6]. These volatiles are a potential source of information regarding metabolism and disease and were routinely used by medical practitioners in past centuries for their diagnostic value [7–10]. In the present era, disease-related VOCs are once again being considered for their potential in diagnosis and monitoring of disease due to the development of efficient methods for collection of volatile organic compounds as well as efficient and sensitive techniques for separation and identification of complex mixtures, such as gas chromatography and combined gas chromatography–mass spectrometry (GC/MS) [11–13]. Although GC/MS is not well suited for "near-patient" use, the technique holds great potential to identify volatiles characteristic of a disease process and/or pathway that can be exploited for detection by sensor-based electronic recognition methods [8,14–18].

A number of investigations have focused on human breath analysis for volatile compounds indicative of oral [19–21] and systemic [18,22–31] pathologies. Exhaled air may be collected in a relatively non-invasive manner, with minimal inconvenience for the patient [32–34]. Part of the volatile mix in exhaled nasal and/or oral air may include compounds originating in the nasal sinuses, particularly when they are infected [35].

Sinus infection or, "sinusitis," is an extremely common medical diagnosis, affecting 1 in 8 people over the course of a lifetime [36]. Sinusitis is diagnosed in 31 million individuals in the United States annually and is the most common of all health care complaints, affecting 14.1% of the U.S. population [37]. Acute bacterial sinusitis occurs in 0.5–2% of these cases [38,39]. Sinusitis is the most frequently reported chronic medical condition.

The diagnosis of sinusitis can be difficult to make, as it may be confused with a host of other nasal conditions. In addition, there is no single standard for diagnosis of sinusitis. Typically, diagnosis is made by clinical criteria combined with the identification of bacte-

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rial pathogens in cultures of secretions from the involved sinuses. Chief among these clinical criteria are facial pain, facial fullness, nasal obstruction and blockage as well as purulent discolored nasal discharge [40].

These criteria, coupled with results from positive patient cultures, represent our closest approximation to a "gold standard" of diagnosis. Studies have shown that 76% of acute sinusitis cases are caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* [41]. Other species, including *Branhamella* (*Moraxella*) *catarrhalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*, are also significant contributors to acute sinusitis or acute exacerbation of chronic sinusitis. A more rapid and accurate diagnosis of bacterial sinusitis, would assist physicians in determining which patients require antibiotics, guide selection of the most appropriate antibiotic and avoid antibiotic use in those patients not experiencing bacterial infections.

Since infected sinus mucus has not been analyzed using GC/MS techniques, we hypothesized that GC/MS could be used to identify volatiles characteristic of the predominant bacterial species responsible for sinus infections in patients. Knowing the structures of the characteristic volatiles will provide a guide for creation of compound-specific sensors [42–44]. Fitted to electronic nose ("enose") devices, sensors responding to bacterial volatiles [45–49] would aid physicians in choosing antibiotics in real time, similar to the rapid strep test used for acute pharyngitis [50]. A commercially available enose device has been applied to ear and sinus infections [27], ventilator associated pneumonia [51], as well as asthma [18]. The sensors in this device comprise an array of 32 chemiresistors [52]. Use of volatile-specific detectors may enhance speed, selectivity and specificity of disease diagnosis [44].

Previous analyses of volatiles from bacterial species of relevance to sinus disease have been performed [53–56]. In these studies, dynamic headspace collection of volatiles above bacteria cultures was followed by thermal desorption. GC and GC/MS were used to separate and identify compounds from cultures of *P. aeruginosa*; these included 2-nonanone, 3-undecanone, 2-amino-acetophenone, dimethylsulfide, dimethyltrisulfide, butanol, 2-butanone, 1-undecene and isopentanol [57,58]. A number of other VOCs have been identified from upper respiratory pathogens. In order to better characterize the volatiles associated with bacteria involved in sinusitis, we employed solid-phase microextraction (SPME) [59–61] to collect headspace volatiles above standard cultures as well as above samples of infected sinus mucus. Subsequent separation and identification of the collected VOCs were done using GC/MS as well as GC with flame photometeric detection. The results reported in this "proof-of-concept" study suggest that the volatiles characteristic of certain infecting organisms can be clearly identified in the recovered sinus mucus samples.

2. Experimental

2.1. Solid-phase microextraction

The solid-phase microextraction fibers used for collection of bacterial and sinus mucus odors were 2 cm, 50/30 μm divinylbenzene/carboxen on polydimethylsiloxan (DVB/CAR/PDMS Stableflex fibers, Supelco Corp., Bellefonte, PA).

2.2. Bacteria of relevance to sinus disease

Bacteria commonly associated with acute and chronic bacterial sinusitis were chosen and sampled from plated specimens, prepared by the Microbiology Laboratory at the Hospital of the University of Pennsylvania. These specimens are routinely kept as reference organisms, and were typically grown in favorable media for at least 48 h. Bacteria sampled included *Streptococcus* (*Strep.*) *pneumonia*, *Haemophilus* (*H.*) *influenzae*, *Pseumdomonas* (*P.*) *aurginosa*, *Staphylococcus* (*Staph.*) *aureus*, *Moraxella* (*M.*) *catarrhalis* and *Stenotrophomonas* (*S.*) *maltophilia.*

Petri dishes with either blood agar (ba) or chocolate blood agar (cba) were used to obtain optimal bacterial growth prior to sampling the volatile profile from each culture. In addition, the volatiles found above dishes with only these growth media were also examined.

2.3. Sampling headspace of bacteria cultures

A hole was drilled in the side of the 9 cm plastic Petri culture dish and a Restek IceBlue® septum placed between the hole in the side of the top of the dish and the hole in the side of the bottom of the dish to both maintain sterility and provide access for the odor-sampling SPME fiber. The culture dish was held at room temperature prior to insertion of the 3-phase fiber through the septum using the SPME applicator. The fiber was exposed for 30 min prior to GC/MS analyses. This enabled the production and identification of characteristic volatiles from each of the putative infectious bacteria, as identified in Table 1.

2.4. Collection and culture of sinus mucus samples

The material for culture and analytical studies was obtained in patients suspected of having acute sinusitis or an acute flare of chronic sinusitis as judged by clinical criteria. Samples were collected trans-nasally, under endoscopic visualization after topicalization of the patients' nares with aerosolized 2% pontocaine and 1% ephedrine. Aliquots of mucus from each patient were used for both the analytical measurement of headspace volatiles and bacterial culturing. The bacteria in the mucus were identified by standard culture technique of plating secretions sampled from the sinuses endoscopically. This was done in the Microbiology Laboratory at the Hospital of the University of Pennsylvania. The methods employed for bacteria identification are standard in clinical practice.

All patients' nasal cavities were sprayed with topical anesthetic (pontocaine and ephedrine, as described above) and then examined with a rigid nasal endoscope. If there was extractable mucopurulence in a sinus cavity or at a site of drainage from the sinus cavity, then this material was removed under endoscopic visualization and sent to culture via standard culture swab and for GC/MS analysis.

2.5. Sampling of sinus mucus volatiles

The samples of sinus mucus used for analytical studies were collected into 4 ml glass vials and typically spread on the sides of the vial. The vials were fitted with septum caps for SPME sampling. Vials were placed in a constant temperature water bath at 37 °C and incubated for 15 min prior to placement of the SPME fiber through the septum in the cap of the vial. The fiber was left exposed to headspace volatiles for 30 min prior to removal to the GC/FPD or GC/MS system (see below). Separate collections from the same sinus sample were performed for each of the analytical methods; volatiles for analysis by GC/MS were always collected first. Following the collection of sufficient volatiles for instrumental analyses the amount of sinus mucus in several of the vials ($n = 8$) was calculated by weighing the vials with sinus mucus. After obtaining this measure, the mucus was removed with a cotton-tipped swab and the vial weight minus mucus was obtained.

Table 1
Volatile organic compounds produced by microorganisms in standard culture media.

Microorganisms	Characteristic compounds: in order of abundance
Streptococeus pneumoniae (*Strep. pneumonicae*)	Benzaldehyde, benzylalcohol, 2-phenylethyl alcohol, acetic acid and methyl mercaptan
Haemophilus influenzae (*H. influenzae*)	Indole, benzaldehyde, acetic acid and benzylalcohol
Branhamella (Moraxella) *catarrhalis* (*B. cattarrhalis*)	Benzaldehyde, benzylalcohol and 2-phenylethyl alcohol
Staphylococcus aureus (*Staph. aureus*)	Isovaleric acid, 2-methylbutyric acid, isobutyric acid, 1-hydroxy-2-propanone, 3-hydroxy-2-butanone, butyric acid, 4-methylhexanoic acid and 2-phenylethyl alcohol
Pseudomonas aeruginosa (*P. aeruginosa*)	2-Amino-acetophenone, dimethyldisulfide, undecene, dimethylpyrazine and dimethylsulfide
Stenotrophomonas maltophili (*S. maltophilia*)	Methylpyrazine, dimethylpyrazine, trimethylpyrazine 2-dodecane, α,α′-dimethylbenzene methanol, acetophenone, 2-phenylethyl alcohol and caprolactam

2.6. Gas chromatography with flame photometric detection (GC/FPD)

Analyses using GC with FPD were performed in order to help to elucidate the presence of volatile sulfur compounds in either bacterial cultures or sinus mucus. The FPD is both highly specific and sensitive to the presence of sulfur-containing organic compounds. For these analyses we employed a Finnigan 9001 Gas Chromatograph fitted with a FPD. The filter on the FPD was set for 394 nm to detect only sulfur emissions. Injections of standard solutions of dimethyldisulfide demonstrated detection levels of ≤0.001 ng. The Stabilflex, 3-phase SPME fiber containing the sample for analysis was inserted into the chromatograph's injection port and desorbed for 3 min at 225 °C. A Stabilwax column (30 M × 0.32 mm with 1.0 μm coating; Restek Corp., Bellefonte, PA) was employed for separation. For GC studies, the injection was performed at an initial temperature of 60 °C; after a 4 min hold, the column was heated at a rate of 6 °C/min to a final temperature of 220 °C and kept there for 30 min; total run time was approximately 61 min.

2.7. Gas chromatography/mass spectrometry (GC/MS)

A Thermoquest/Finnigan Voyager GC/MS with Xcalibur software was used for all analyses. A polar, Stabilwax column, 30 M × 0.32 mm with 1.0 μm coating (Restek Corp., Bellefonte, PA) was used for separation and analysis of the SPME-extracted volatiles from samples. The separation of volatile components used the following protocol: after a 60 °C hold for 4 min, the chromatograph's oven was programmed at 6 °C/min to 220 °C with a 30 min hold at this final temperature. The injection port is held at 230 °C. Helium carrier gas was used at a constant rate of 2.5 ml/min.

Data acquisition and operating parameters for the mass spectrometer were as follows: scan rate 2/s; scan range *m*/*z* 33 to *m*/*z* 400; ion source temperature 200 °C, and ionizing energy at 70 eV.

Identification of compounds was done using both the NIST' 02 library as well as manual comparison of mass spectra with those reported in the literature. In addition, comparisons of relative retention times and mass spectra of commercially available standards were performed when available. Standard chemicals for structure and retention time confirmation were obtained from Sigma–Aldrich, St. Louis, MO.

3. Results

3.1. Analyses of volatiles from bacterial cultures

Prior to analyses of infected mucus samples from patients, analyses of headspace volatiles from empty plastic Petri dishes as well as Petri dishes containing culture media not inoculated with bacteria were analyzed (Fig. 1). These analyses consistently showed ethanol, toluene, styrene, 2-ethyl-1-hexanol, butylated-hydroxytoluene, and background methyl siloxanes as well as other compounds which most likely originated from the culture media, plastic, septum and SPME fiber degradation products. These samples lacked the bacteria-related volatiles seen in cultures described below.

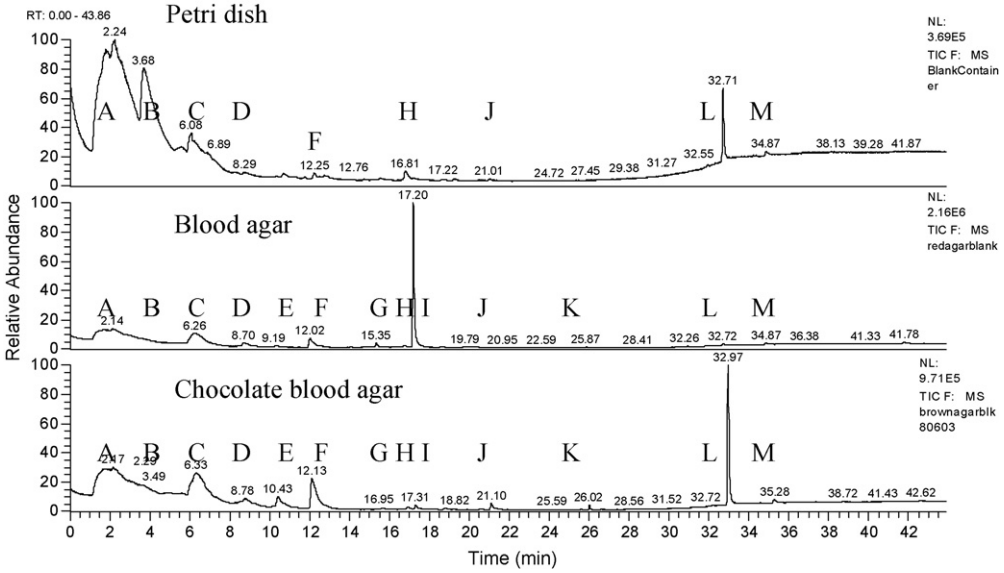


Fig. 1. Total ion chromatograms (TICs) of empty Petri dish as well as Petri dishes containing only the two media used to grow the standard bacteria described in the text. The *x*-axis on all TICs shows increasing retention time (in min) while the *y*-axis for all TICs shows the relative abundance (from 0 to 100). A = methyl siloxanes; B = ethanol; C = toluene; D = butanol and xylenes; E = pentanol; F = styrene; G = methylcyclopentanol; H = acetic acid; I = 2-ethyl-hexanol; J = butyrolactone; K = butylated hydroxytoluene; L = L-cyclohexyl-2-pyrrolidine; M = benzoic acid.

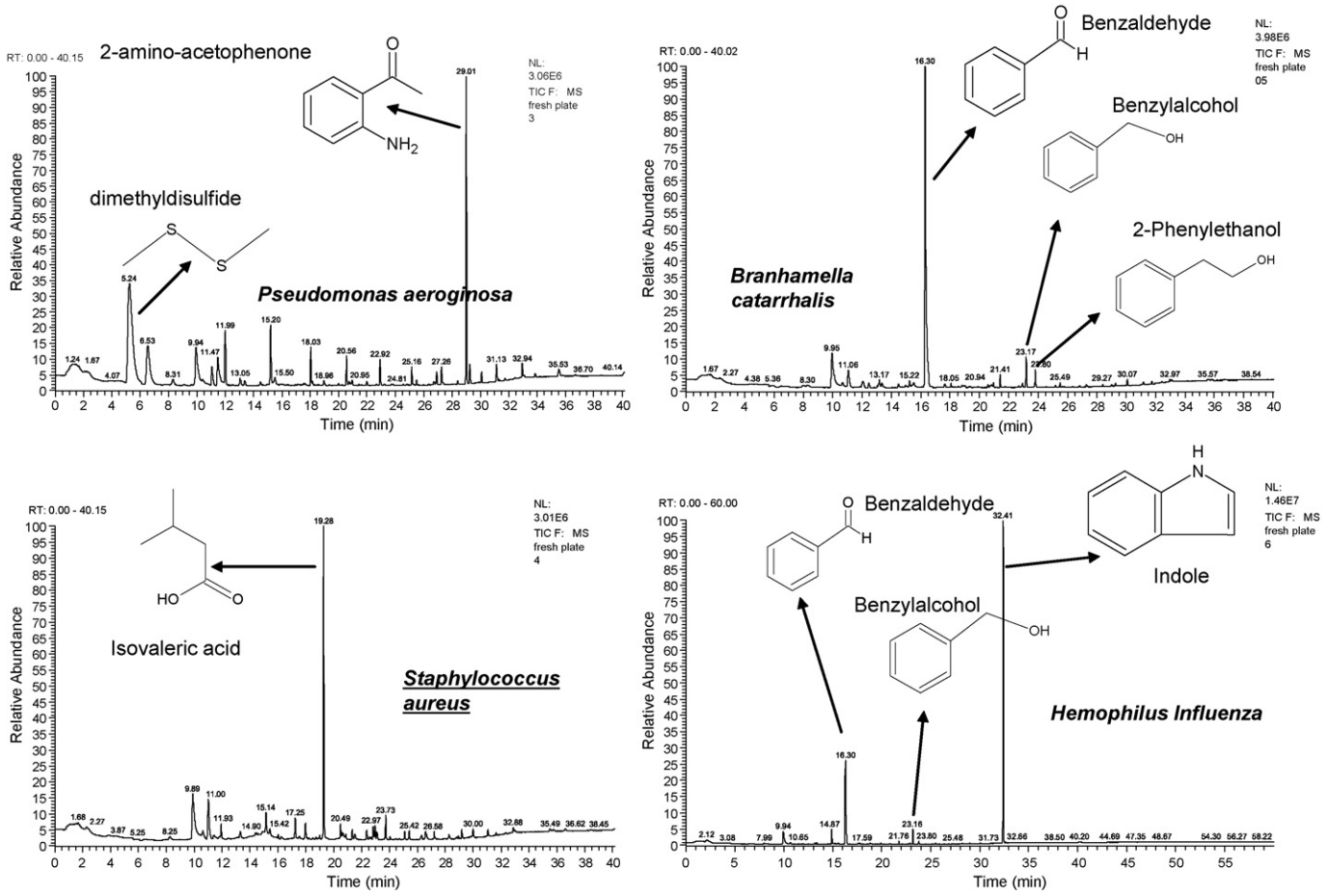


Fig. 2. SPME-collected volatiles from cultures of four bacteria frequently isolated from the sinus mucus collected from patients with sinusitus. The *x*-axis on each of the four TICs shows increasing retention time (in min) while the *y*-axis for all TICs shows the relative abundance (from 0 to 100). The major volatiles found in the headspace above each culture are indicated. Minor components are not identified but many are listed in Table 1.

Six bacterial species of relevance to sinusitis were grown on either ba or cba to assess the volatiles produced by each bacterium: *S. pneumonia* (on ba), *H. influenzae* (on cba), *P. aeruginosa* (on ba), *Staph. aureus* (on ba), *M. catarrhalis* (on cba) and *S. maltophilia* (on cba). Volatile compounds collected by SPME were desorbed and analyzed by GC/MS analysis. Typical total ion chromatograms (TICs) for headspace VOCs produced by four of the bacterial species are shown in Fig. 2. The bacteria shown are *Staph. aureus* and *P. aeruginosa* as well as *H. influenzae* and *M. catarrhalis*. The designated growth media generally yielded the greatest abundance of volatiles for that particular bacterium. The figure also shows that for each bacterium, one compound is particularly abundant and characteristic. The major compounds that characterize each of the cultured bacteria are shown in Table 1. Other minor components

Table 2
Gram stain and bacterial culture results from each patient's sinus mucus.

Vial ID#	Culture site	Organism	Gram stain
Patient 1	L. Ethmoid	Many *Staph. aureus*	Moderate Gram positive cocci' Few WBC's
Patient 2	L. Max Sinus	Many group B *Strep.* Moderate *Enterococcus* species Rare *Staph. aureus*	Moderate Gram positive cocci Few WBC's
Patient 3	L. Middle Meatus	Few *P. aeruginosa*	Rare Gram negative rods Rare WBC's Moderate Epithelial Cells
Patient 4	L. Ethmoid Sinus	Moderate alpha-hemolytic *Strep.*	Moderate Gram positive cocci Many WBC's Few Epithelial Cells
Patient 5	R. Middle Meatus	Many *H. influenzae*, beta-Lactamase + Moderate *P. aeruginosa* Few *Staph. aureus* Mixed Upper Respiratory Flora	Many Gram negative rods Many WBC's Few Epithelial Cells
Patient 6	R. Ethmoid	No growth	Many Polymorphonuclear Leukocytes No bacteria seen
Patient 7	L. Middle Meatus	Rare *Enterobacter Cloacae*	Rare Gram negative rods Rare WBC's
Patient 8	L. Middle Meatus	Moderate *H. influenzae* Beta Lactamase negative	Rare Gram negative rods Rare WBC's
Patient 9	L. Middle Meatus	Few *Staph.* species (coagulase negative) Rare alpha-hemolytic *Strep.*, (not enterococcus or *Strep. pneumonicae*)	No bacteria seen Moderate WBC
Patient 10	R. Ethmoid	No growth	No bacteria seen Few WBC Few Epithelial Cells
Patient 11	R. Maxillary	Many *P. aeruginosa*	Moderate polymorphonuclear leukocytes; few Gram negative rods
Patient 12	R. Maxillary	Few *Acinetobacter baumannii*, often appears as Gram positive cocci	Many WBC's

Abbreviations used: R = right; L = left; WBC = white blood cell.

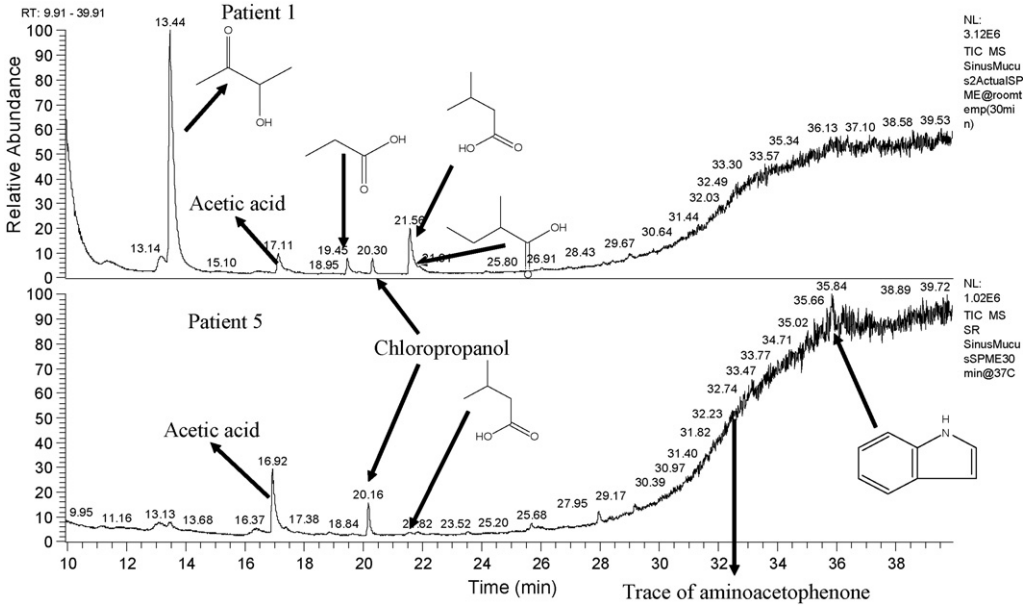


Fig. 3. SPME-collected volatiles from sinus mucus collected from 2 patients, described in Table 2. The chloropropanol is seen in all sinus mucus samples since it is found in the nasal anesthetic used during the sinus exams and mucus collection. The *x*-axis on the two TICs shows increasing retention time (in min) while the *y*-axis for each of the TICs shows the relative abundance (from 0 to 100).

are indicated. Benzylalcohol and 2-phenylethyl alcohol are seen to be common to the headspace above several of the bacteria cultures.

3.2. Sinus mucus

The amount of nasal mucus used for analytical studies varied across patients ($n = 12$). An average of 41.72 mg (range = 4.4–133 mg) of mucus was obtained based on sample weights from 8 of the 12 patients. Gram stain results and the bacteria grown from cultures of patients' sinus mucus are shown in Table 2.

3.3. Analysis of volatiles from sinus mucus

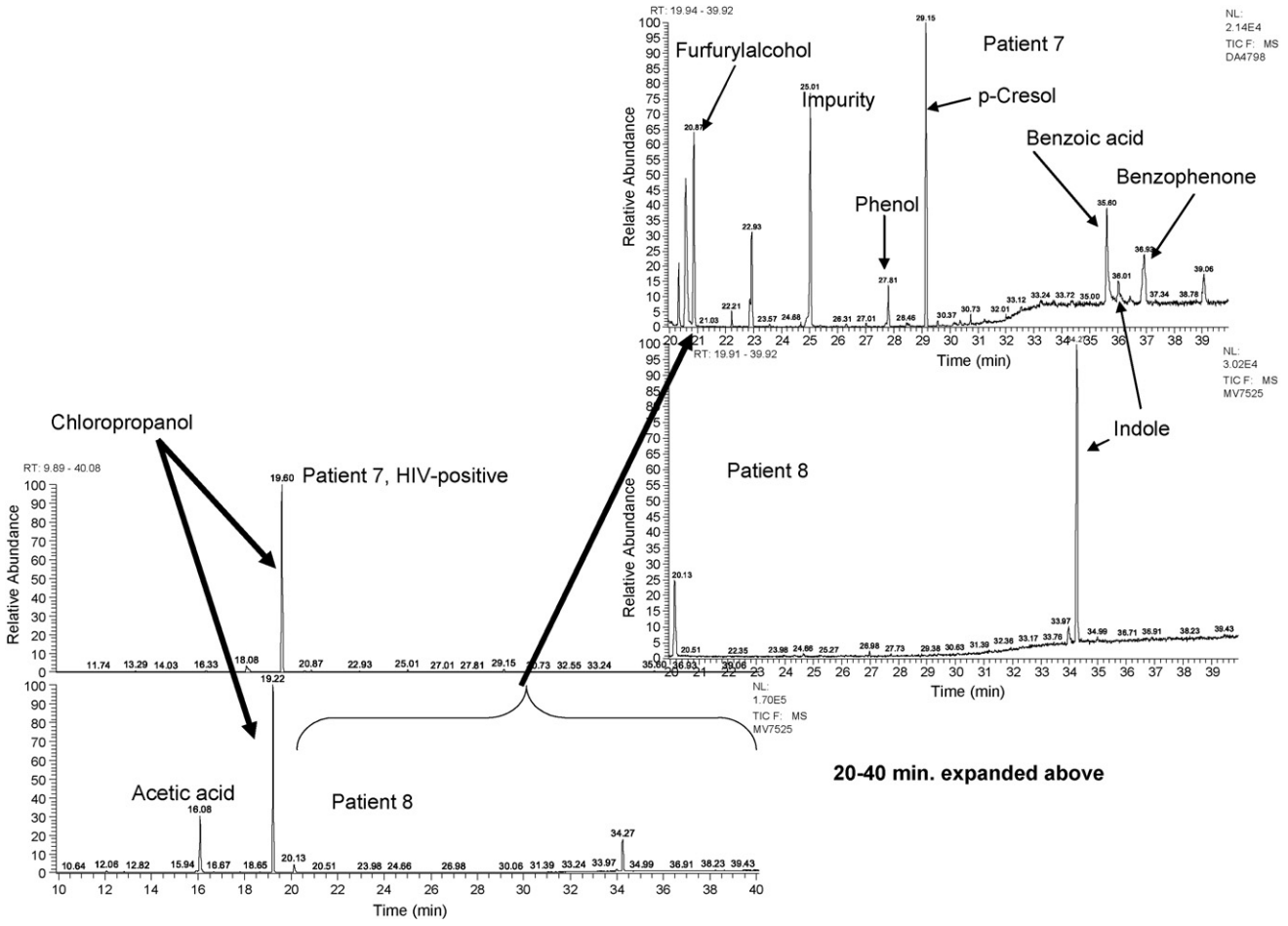
Bacterial cultures of the patient-derived mucus samples demonstrated that most samples were a mixture of flora but several samples were dominated by one species. However, two samples from Patients 6 and 10 revealed "no growth." Patients 1 and 2 had similar flora dominated by Gram positive rods, principally *Staph. aureus* and beta-hemolytic *Strep*. The mixed flora from Patient 5 was unique since it contained both *H. influenza* and *P. aeruginosa*. Bacterial flora from Patients 3 and 11 also contained *P. aeruginosa*; however, the flora from Patient 3 appeared to contain a relatively smaller amount than the culture from Patient 5. A moderate amount of alpha-hemolytic *Strep.* was found in the sample from Patient 4. Patient 7 exhibited unusual Enterobacteria in his sinus mucus; clinical data from this patient also showed that he was infected with HIV. Bacteria grown from the mucus of Patient 12 contained *Actinetobacter baumannii*, a bacteria that we had not previously cultured on standard media.

GC/MS analyses demonstrated the presence of trichloropropanol (henceforth chloropropanol) in varying amounts, in all the patient samples (but not in cultures of bacteria on standard media). This compound comes from topical anesthetic products used to prepare the patient prior to mucus collection and is indicated in the TICs shown in Figs. 3 and 4. Our analyses also demonstrated that seven of the samples produced responses from the FPD: those from Patients 3, 4, 6, 7, 8, 11 and 12. Both the mass spectra and retention time data from the GC/MS system from these patients demonstrated that the sulfur-containing compounds responsible for these responses were dimethylsulfone in Patient 3; hydrogen sulfide in Patients 7, 11 and 12 and dimethylsulfide in Patients 6, 8 and 11. Hydrogen sulfide and dimethylsulfone were not seen in cultures of pure bacteria; however, dimethylsulfide was seen in the VOCs from *P. aeruginosa*, albeit very low amounts (~3% of the most intense compound, 2-amino-acetophenone) and methylmercaptan was seen in the volatile profile from *Strep. pneumonicae*.

Fig. 3 shows the TICs from mucus samples of Patients 1 and 5, which differed in their bacterial make-up, as noted above. The flora from Patient 1 is dominated by *Staph. aureus* and produces isovaleric acid and other short-chained volatile acids [e.g. propanoic acid, 2-methylbutyric acid and isobutyric acid (not labeled in Fig. 3)]. These acids were also seen in the culture of pure *Staph. aureus.* Mucus from Patient 5 showed indole and a trace amount of isovaleric acid, (seen by searching for m/z 60, its mass spectrum base ion) both indicative of the *H. influenzae* and *Staph. aureus*, respectively. Both of these bacteria were identified in the mucus by culture; in addition, *P. aeruginosa* appears to contribute a trace amount of 2-amino-acetophenone, visible only by examining the single-ion chromatogram indicative of the molecular ion (m/z 135). The retention time is also compatible with the elution time of the 2-amino-acetophenone standard. Acetoin (3-hydroxy-2-butanone), the large peak seen at 13.44 min in Patient 1, appears to be indicative of the flora found in this patient and that of Patient 2 (data not shown). However, acetoin was not a large component in the headspace above pure cultures of *Staph. aureus*; it is seen as a very small peak at 10.61 min in the TIC corresponding to *Staph. aureus* in Fig. 1.

The presence of indole in the volatiles from Patients 5, 7, 8, 10 and 11 suggested that *H. influenza* would be present in mucus samples from these patients. This was confirmed by the culture results only for Patients 5 and 8.

Fig. 4 shows the TICs generated by the analyses of Patients 7 and 8. Both had large amounts of chloropropanol. Since the TICs are presented in a normalized fashion, we present the normalized portion of each of these two samples from 20 to 40 min on the right-hand side of Fig. 4 to demonstrate other components present which were suppressed by the intensity of the chloropropanol. Patient 7 is the HIV-positive individual whose mucus contained



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Gram negative rods identified as *Enterobacter cloacae*. In addition to indole, the volatiles from the mucus of this patient also contained p-cresol and benzophenone. Bacteria grown from the mucus from Patient 12 demonstrated the presence of *A. baumannii*, an organism which we had not previously examined for characteristic volatiles. However, the SPME–GC/MS analysis of this sample revealed the presence of hydrogen sulfide but no other distinguishing VOCs, except for traces of pyridine and acetamide were identified. A summary of the compounds identified in each mucus sample is shown in Table 3.

Table 3
Compounds observed in GC/MS analyses of patients' sinus mucus.

Patient ID	Compounds observed in GC/MS analysis: listed by elution time (early to late)	Organism
Patient 1	3-Hydroxy-2-butanone, acetic acid, propanoic acid, isobutyric acid, isovaleric acid, 2-methylbutyric acid, caprolactam and phenol	Many *Staph. aureus*
Patient 2	3-Hydroxy-2-butanone, acetic acid, isobutyric acid, isovaleric acid, 2-methylbutyric acid, benzyl alcohol and phenol	Many Group B *Streptococcus* Moderate *Enterococcus* Rare *Staph. aureus*
Patient 3	Acetone, dimethylsulfone, phenol	Few *P. aeruginosa*
Patient 4	Acetone, dimethylsulfide, butan-1-ol, dimethylsulfone and phenol	Moderate alpha-hemolytic *Strep.*; no *Enterococcus*
Patient 5	Acetone, 3-hydroxy-2-butanone, acetic acid, propanoic acid, traces of isovaleric and 2-methylbutyric acid, phenol and indole. Trace of 2-amino-acetophenone	Many *H. influenzae*, Moderate *P. aeruginosa* Few *Staph. aureus* Mixed Upper Respiratory Flora
Patient 6	Dimethylsulfide, butan-1-ol	No growth
Patient 7	Hydrogen sulfide, acetone, butan-1-ol, butyrolactone, furfuryl alcohol, acetamide, phenol, p-cresol, p-ethylphenol, benzoic acid, indole and benzophenone	Rare *Enterobacter cloacae*
Patient 8	Dimethylsulfide, acetone, acetic acid, butyrolactone, indole	Moderate *H. influenzae* Beta Lactamase negative
Patient 9	Very weak sample, few compounds seen. Low levels of C_2–C_{10} organic acids, trace of pyridine and phenol.	Few *Staph*. species (coagulase neg.) Rare alpha-hemolytic *Strep*., (not *Strep. pneumo*)
Patient 10	Considerable siloxanes from fiber or septum degradation; however, acetic acid, caprolactame and indole clearly present all at similar abundance.	No growth
Patient 11	Hydrogen sulfide, dimethylsulfide, trace of dimethyldisulfide, acetamide and trace of indole.	Many *P. aeruginosa*
Patient 12	Hydrogen sulfide, pyridine, acetic acid, acetamide	Few *Acinetobacter baumannii* isolated from this culture (appears as Gram + cocci on direct smears).

4. Discussion

Our results demonstrate that the volatile mixtures from the sinus mucus samples that contained certain bacteria differed in composition from pure cultures of those particular bacteria. Data presented here suggest that identification of bacteria-specific volatiles from a sample of infected mucus is possible in certain cases, even if small amounts of mucus are present. In those samples where *Staph. aureus* and *H. influenzae* were the dominant bacteria grown from the mucus (e.g. Patients 1, 2, 5 and 8), we found that many of the characteristic VOCs seen in pure cultures of these bacteria were emitted from the infected sinus mucus. We also noted that the ratio and types of volatiles produced from sinus samples most likely differs in the natural environment (mucus) due to different growth substrates as well as other competing organisms. For example, the mucus samples containing *Staph. aureus* contained enhanced levels (vs. pure cultures) of certain volatiles, such as acetoin, which may be indicative of growth in the sinus environment. Consequently, although identifying the bacteria-specific volatiles is aided by our knowledge of the compounds produced in pure cultures, our purpose might be better served if bacteria could be cultured with sinus mucus to more closely simulate the sinus environment. This may be particularly true in the case of mucus containing *P. aeruinosa*, or *Strep. pneumonicae*; no patient-related mucus contained *S. maltophilia* or *M. cattarrhalis*.

Procedure-derived artifacts (e.g. chloropropanol) were also present, and in certain samples, were the dominant volatile present, as seen in Fig. 4. Samples containing *P. aeruginosa* yielded trace levels of 2-amino-acetophenone (Patient 5) or dimethyldisulfide (Patient 11); these compounds were detected in the baseline by searching mass chromatograms of important ions. These compounds are prominent and characteristic of the volatiles from pure cultures of this microbe as reported above, in this study, and in prior research [45,46]. In addition, neither the VOCs above the culture media nor the pure bacteria grown on these media, demonstrated the presence of hydrogen sulfide (Patients 7, 11 and 12) or dimethylsulfide (Patients 4, 6, 8 and 11) seen in the mucus from patients.

Patients 6 and 10 showed no growth in their cultures, suggesting no pathogenic bacteria were present. In the absence of bacteria growing from culture it is virtually impossible to assign causation to a sinus infection. Consequently, for the 2 patients whose cultures showed no growth, it is theoretically possible that a viral infection was present. But it is also possible that another non-infectious, inflammatory process was at work, for example, seasonal allergy.

The type and abundance of VOCs seen in our analyses appeared to be independent of the amount of mucus collected. For example, the largest amount of mucus collected from Patient 10 (approx. 133 mg), yielded no bacterial growth and no distinguishing VOCs. However, the many siloxanes seen (see Table 3) may have indicated a failure of the fiber or excessive septum bleed. Sinus mucus collected from Patients 6 and 9 were two of the smallest mucus samples collected (approx. 4.4 mg each). The sample from Patient 9 yielded few bacteria and no VOCs indicative of *Staph. aureus*.

The HIV-positive patient demonstrated that bacteria unrelated to those normally associated with sinus infections (in non-HIV patients), may yield volatiles indicative of certain sinus-related bacteria. In this patient, indole appeared to be produced by the enterobacterium present and not by *H. influenzae*. This example suggests that, regardless of analytical techniques used, cultures may have to be performed, in certain cases, concomitantly with analytical instrument-based diagnoses.

More than 51 million doctor visits were recently recorded annually for patients presenting with upper respiratory tract infections and "head colds". Since a diagnosis relying solely upon bacterial cultures may take up to 48 h, with further time necessary to determine antibiotic sensitivity, many physicians often prescribe antibiotics presumptively, resulting in over-prescription of antibiotics and a widely noted increase in drug-resistant microorganisms [62,63]. A government study showed that of 51 million visits to physicians for "colds," upper-respiratory infections and bronchitis in the United States, 50–66% resulted in an antibiotic prescription (Current Estimates From the National Health Interview Survey: United States, 1985. Moss, A. J., Parsons, V. L. December 1986. 192 pp. (PHS) 86-1588. PB87-125851. PC A09 MF A02). A reliable, fast, patient-side diagnostic tool based upon volatiles emitted by growing microorganisms would provide clinicians with more information with which to correctly diagnose the absence or presence of one or more infectious organisms.

5. Conclusion

Electronic nose-based sensor arrays have been shown capable of distinguishing among various pure cultures of bacteria responsible for eye, sinus and respiratory infection These devices can be sufficiently sensitive to identify bacteria at the sub-species and strain level. The results presented here, however, demonstrate the importance of substrate and environment. VOCs from single, pure bacteria cultures grown on standard media differ in either relative abundance or type from sinus mucus containing the same bacteria. Sinus mucus and an individual's sinuses undoubtedly provide a different mixture of nutrients and available oxygen as well as competition among organisms for these items; these are far different factors than standard laboratory conditions. This type of knowledge is important for determining whether or not specific sensors for electronic olfaction and hence detection of disease or organism-specific volatiles can be created; these are important goals of this research.

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