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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.
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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.

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