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Identification of Upper Respiratory Bacterial Pathogens With the Electronic Nose

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Objective: **To use an electronic nose to identify common upper respiratory bacterial pathogens.** ***Study Design:*** **Controlled *in vitro* analysis.** ***Methods:*** **Swabs of bacteria were obtained from *in vitro* samples. The specimens were vaporized and analyzed over the organic semiconductor-based electronic nose (Cyranose 320). Data from the 32-element sensor array were subjected to principal component analysis for depiction in two-dimensional space and differences in odorant patterns were assessed by calculating Mahalanobis distances.** ***Results:*** **The electronic nose was able to distinguish between control swabs and bacterial samples. Furthermore, calculation of the Mahalanobis distances among the various bacteria demonstrated distinct odorant classes (Mahalanobis distance ⩾3). This demonstrates that the electronic nose could differentiate among various common bacterial pathogens of the upper respiratory tract, including *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenza*, and *Pseudomonas aeruginosa*.** ***Conclusions:*** **The electronic nose represents a novel method to identify potential upper respiratory infections and to discriminate among common upper respiratory bacterial pathogens. This technology could provide a rapid means to identify organisms causing upper respiratory infections.** ***Key Words:*** **Electronic nose, bacteria, sinusitis.**

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INTRODUCTION

Technological advances in the last decade have created several technologies that reproduce the ability of biologic olfactory systems to analyze volatilized molecules with remarkable precision.[1] While previous technologies relied on odor-specific sensors, a recently developed electronic nose technology is based on resistance changes of organic chemoresistors that interact with volatile odorant molecules. An array of different organic polymers that respond to specific stereochemical characteristics of an odorant is used in a single device. Multidimensional data obtained from the sensor array may be processed by neural networks and/or principal component analysis to discriminate and identify odorant samples.[2,3]

Although there is anecdotal evidence of particular odors associated with specific disease processes, little scientific data exists. Furthermore, while electronic nose technology has been used in industrial applications, such as the detection of food spoilage, only preliminary attempts have been made to use this technology clinically. Infection of leg ulcers with beta-hemolytic streptococcus can be differentiated from uninfected leg ulcers.[4] Analysis of exhaled gases can distinguish patients with pneumonia from control subjects.[5] More recently, a preliminary investigation demonstrated the ability of organic polymer-based electronic nose technology to distinguish between cerebrospinal fluid and serum.[6]

These preliminary applications demonstrate the potential usefulness of electronic nose technology in many different clinical settings. To determine the usefulness of this technology in the detection of upper respiratory infections by bacterial pathogens, we evaluated the ability of the electronic nose to detect the presence or absence of bacteria on a culture swab. Following the detection of bacteria in a given sample, we assessed the ability of this technology to distinguish among different bacterial species.

MATERIALS AND METHODS

Known samples of a variety of bacterial isolates were obtained from the William Pepper Laboratory of the University of Pennsylvania. Samples were prepared by using a sterile Nasopharyngeal Calcium Alginate Tipped Applicator (Pur-Wraps Calgiswab type 1; Hardwood Products Co. LLC, Guilford, ME); to

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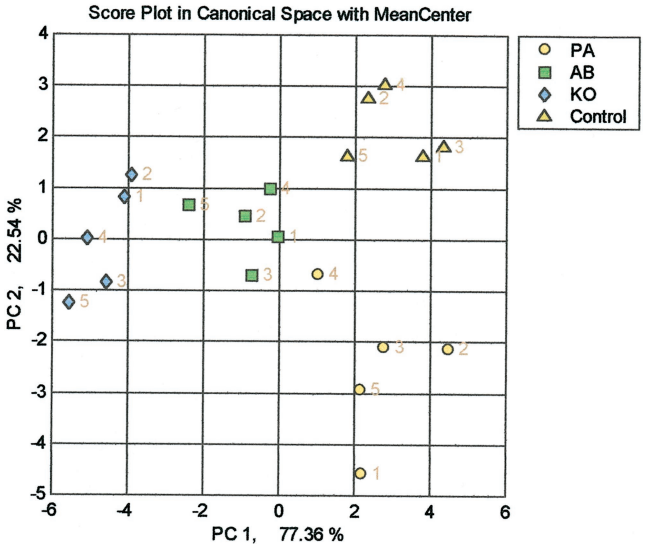

Fig. 1. Comparison of bacterial samples with control swabs using all sensors of the electronic nose. Mean-centered canonical plot projection. Yellow circle = *Pseudomonas aeruginosa* (PA); green square = *Acinetobacter baumanii* (AB); blue diamond = *Klebsiella oxytoca* (KO); yellow triangle = control.

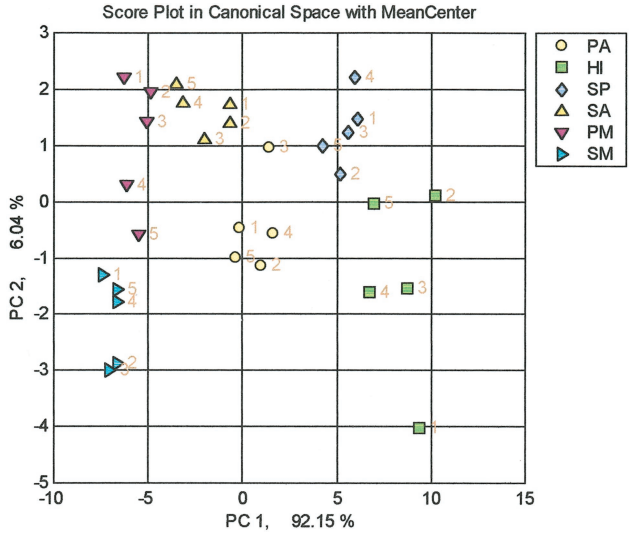

Fig. 3. Discrimination of bacterial samples by the electronic nose (I). Mean-centered canonical plot projection. Yellow circle = *Pseudomonas aeruginosa* (PA); green square = *Haemophilus influenza* (HI); blue diamond = *Streptococcus pneumoniae* (SP); yellow triangle = *Staphylococcus aureus* (SA); purple triangle = *Proteus mirabilis* (PM); blue triangle = *Stenotrophomonas maltophilia* (SM).

sample a colony of the bacterial growth. Colonies less than 6 mm in diameter were swabbed to saturate the head of the Calgiswab without digging into the culture material. The Calgiswab was then placed in a 20-cc glass headspace vial (VWR) and 0.3 mL of sterile, normal saline solution was added. The vial was sealed with an aluminum seal surrounding PTFE/silicon septa (VWR) using a 20-mm Kimble Hand-Operated Crimper (VWR; Kimble, Vineland, NJ). Control or calibration samples were prepared by placing a fresh Calgiswab into a headspace vial with 0.3 mL of normal saline solution. The prepared vials were incubated in a dry heater block set at 39°C for at least 30 minutes. The headspace vial temperature was 36° ± 2°C.

Specimens were analyzed by the polymer composite sensor array electronic nose (Cyranose 320, Cyrano Technologies, Pasadena, CA). Collected data was processed using Savitzky-Golay filtering and baseline correction. Different processing methods, such as normalization and scaling, were applied to determine the best discrimination among samples. Modeling was done using principal component analysis (PCA) to reduce the data from 32 individual sensor responses to vectors or prinicipal components.[7] The vectors were calculated to capture variance and the results were plotted in two dimensions to illustrate differences among the bacterial samples. Results from PCA were used in canonical

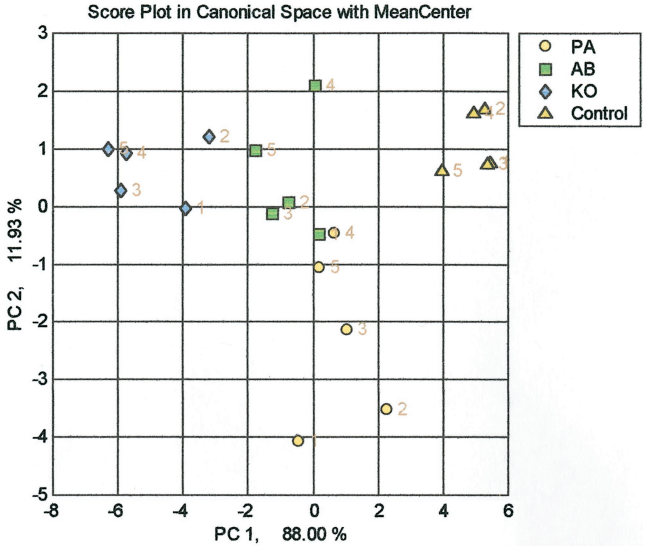

Fig. 2. Comparison of bacterial samples with control swabs using selected sensors of the electronic nose. Mean-centered canonical plot projection. Yellow circle = *Pseudomonas aeruginosa* (PA); green square = *Acinetobacter baumanii* (AB); blue diamond = *Klebsiella oxytoca* (KO); yellow triangle = control.

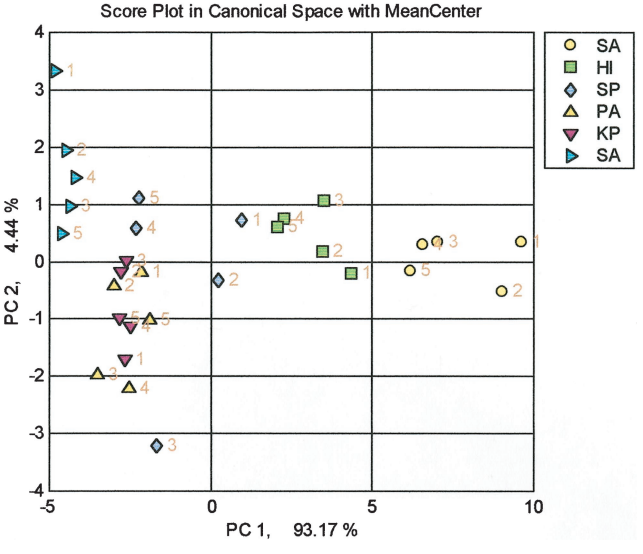

Fig. 4. Discrimination of bacterial samples by the electronic nose (II). Mean-centered canonical plot projection. Yellow circle = *Staphylococcus aureus* (SA); green square = *Haemophilus influenza* (HI); blue diamond = *Streptococcus pneumoniae* (SP); yellow triangle = *Pseudomonas aeruginosa* (PA); purple triangle = *Klebsiella pneumoniae* (KP); blue triangle = *Streptococcus* group A (SA).

TABLE I.
Mahalanobis Distances Between Bacteria Samples and Control Tested in Figure 1.

	Pseudomonas aeruginosa	*Acinetobacter baumanii*	*Klebsiella oxytoca*	Control
Pseudomonas aeruginosa	0	4.4	7.6	4.7
Acinetobacter baumanii		0	3.8	4.3
Klebsiella oxytoca			0	8.0
Control				0

TABLE II.
Mahalanobis Distances Between Bacteria Samples and Control Tested in Figure 2.

	Pseudomonas aeruginosa	*Acinetobacter baumanii*	*Klebsiella oxytoca*	Control
Pseudomonas aeruginosa	0	3.1	6.5	5.4
Acinetobacter baumanii		0	4.3	5.7
Klebsiella oxytoca			0	10.0
Control				0

discriminant analysis (CDA) to create a model to predict unknown samples by maximizing the distance among the different sample classes.

Statistical analysis using standard measures such as *P* values are not applicable to this data analysis. Quantification of the discrimination between two sample classes was performed using the Mahalanobis distance (MD), a measure of class separation between different data clusters.[8] An MD of 3 or greater indicates that the classes are discrete from each other. An MD of 5 or greater indicates that the electronic nose may be able to classify unknown samples belonging to one of several bacterial species in a given model/experiment.

RESULTS

The initial experiment tested the ability of electronic nose technology to distinguish between control and bacterial samples. Five swabs of each bacterial species were compared with five control swabs placed in normal saline. For each of the three bacteria tested, the electronic nose was able to distinguish between the control and bacteria (Fig. 1). The MD ranged from 4.3 for *Acinetobacter baumanii* to 8.0 for *Klebsiella oxytoca* (Table I). Using a select group of the 32 sensors (Fig. 2), the MD increased to between 5.4 and 10 (Table II). Thus, the electronic nose could distinguish between bacterial samples and the controls and potentially identify unknown samples of these bacterial species.

In the next set of experiments, a variety of bacterial species were analyzed to test the ability of electronic nose technology to discriminate among them. Controls were included and could be distinguished from the bacterial specimens as demonstrated above. However, inclusion of the controls in CDA decreased discrimination among bacterial species. Because the initial experiment demonstrated the ability of electronic nose technology to detect the presence of bacteria in a given sample, subsequent analyses focused primarily on the ability of this technology to discriminate among bacterial species.

The first data set included common upper respiratory pathogens, such as *Pseudomonas aeruginosa*, *Haemophilus influenza*, *Streptococcus pneumoniae,* and *Staphylococcus aureus* (Fig. 3). MD of pairwise comparison ranged from 3.4 to 15.4 (Table III). Thus, electronic nose technology was able to distinguish among all the species tested. In 11 of the 15 comparisons, an MD of greater than 5 suggested that the electronic nose technology would be able to identify an unknown specimen given the model established from CDA of this data set.

In the second data set, *Klebsiella pneumoniae* and *Streptococcus* group A specimens were included with the common upper respiratory pathogens from the previous experiment. Again, electronic nose technology could distinguish between species in 12 of the 15 comparisons (Fig. 4). In those cases, the MD was between 3.2 and 12.3 (Table IV). In 7 of those 12 comparisons, the MD greater than 5 implied that the electronic nose could identify an unknown specimen given the model established from CDA of this data set.

TABLE III.
Mahalanobis Distances Between Bacteria Tested in Figure 3.

	Pseudomonas aeruginosa	*Haemophilus influenza*	*Streptococcus pneumoniae*	*Staphylococcus aureus*	*Proteus mirabilis*	*Stenotrophomonas maltophilia*
Pseudomonas aeruginosa	0	7.8	5.2	3.4	6.5	7.8
Haemophilus influenza		0	4.5	10.9	14.2	15.4
Streptococcus pneumoniae			0	7.6	11.2	12.8
Staphylococcus aureus				0	3.7	6.2
Proteus mirabilis					0	3.7
Stenotrophomonas maltophilia						0

DISCUSSION

The objective of this study was to investigate the application of electronic nose technology in the analysis of known *in vitro* bacteria specimens. Specifically, we tested the ability of the electronic nose to distinguish bacterial specimens from controls. Further experiments were directed at testing the ability of the electronic nose to discriminate among different bacterial species.

Clearly, the electronic nose is readily able to distinguish between control samples and small amounts of bacteria on a Calgiswab. In all of our experiments, the bacterial samples had an MD of 3 or greater from the control. Furthermore, the electronic nose could easily distinguish between bacterial species. In the great majority of pairwise comparisons, the MD was 3 or greater. The finding of MD values of 5 or greater suggested that the electronic nose could be used to identify unknown samples given the model established from the CDA of a particular data set.

The ability of the electronic nose to distinguish among bacterial species will require further refinement. In a number of pairwise combinations, the electronic nose was less able to distinguish between bacterial specimens. In the data reported in Figure 4, the electronic nose was least able to distinguish between *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (MD = 1.5); *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* (MD = 2.0); and *Klebsiella pneumoniae* and *Streptococcus pneumoniae* (MD = 2.7). No obvious explanation is evident, particularly because these pathogens belong to different classes of bacteria. Because the electronic nose technology does not rely on odorant-specific sensors, the specific odorant profile of a given bacterial species may be comprised of specific bacterial components or metabolic byproducts. Analysis and comparison of the volatile molecules from a given bacterial species may provide insight into the limitations of the electronic nose technology to distinguish between certain bacterial strains.

The electronic nose establishes odorant profiles of known samples to distinguish bacterial samples and to identify unknown bacterial specimens. The ability of the electronic nose to discriminate among bacterial species appears to depend in part on the given data set. In the pairwise comparison of *Staphylococcus aureus* and *Haemophilus influenza*, the MDs were 4.6 and 10.9 in two different experiments. Similarly, the MDs were 3.4 and 10.4 for *Staphylococcus aureus* and *Pseudomonas aeruginosa,* and the MDs were 2.0 and 5.2 for *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*. In the first two cases, the electronic nose is consistently able at least to distinguish between the species. Interestingly, the electronic nose can differentiate unknown samples of *Pseudomonas aeruginosa* or *Streptococcus pneumoniae* from one another in one experiment, but cannot distinguish the two species in the other data set. These differences may be the result of sample variability within a given data set. Thus, the electronic nose may require an increased number of sample exposures to decrease variance within a given odorant profile. Data collection should improve with increased experience with the electronic nose technology. Additionally, continuing work *in vitro* is focused on decreasing the number of bacteria in the sample to levels comparable with those found *in vivo*.

The ability of the electronic nose not only to distinguish *in vitro* bacterial samples from control swabs, but also to discriminate among bacterial species represents an important step in the use of this technology in a clinical setting. Specifically, this technology could provide a rapid, non-invasive, and inexpensive means to determine the presence or absence of bacterial colonization and/or infection based on sinus swabs or exhaled gases. This could reduce the need for cultures and refine the use of empiric antibiotics, especially in an era of cost-conscious medicine and increasing antibiotic resistance.

CONCLUSION

Electronic nose technology has advanced rapidly with the advent of organic semiconductor arrays. Recent studies have demonstrated the ability of electronic nose technology to determine the presence of bacterial infection. Our *in vitro* studies extend those findings by demonstrating the ability of the electronic nose not only to detect the presence of common upper respiratory pathogens when compared with controls, but also to distinguish among bacterial species. This is an important first step in using electronic nose technology in the detection and diagnosis of upper respiratory infections.

TABLE IV.
Mahalanobis Distances Between Bacteria Tested in Figure 4.

	Staphylococcus aureus	*Haemophilus influenza*	*Streptococcus pneumoniae*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	Streptococcus Group A
Staphylococcus aureus	0	4.6	8.6	10.4	10.5	12.3
Haemophilus influenza		0	4.5	6.0	6.0	7.7
Streptococcus pneumonia			0	2.0	2.7	4.1
Pseudomonas aeruginosa				0	1.5	3.4
Klebsiella pneumonia					0	3.2
Streptococcus Group A						0

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BIBLIOGRAPHY

1. Thaler ER, Kennedy DW, Hanson CW. Medical applications of electronic nose technology: review of current status. *Am J Rhinol* 15:291–295.
2. Kermani BG, Schiffman SS, Nagle HT. A novel method for reducing the dimensionality in a sensor array. *IEEE Trans Instr & Measur* 1998;47:728–741.
3. Kermani BG, Schiffman SS, Nagle HT. Using neural networks and genetic algorithms to enhance performance in an electronic nose. *IEEE Trans Biomed Eng* 1999;46: 429–439.
4. Parry AD, Chadwick PR, Simon D, Oppenheim B, McCollum CN. Leg ulcer odour detection identifies beta-haemolytic streptococcal infection. *J Wound Care* 1995;4:404–406.
5. Hanson CW, Steinberger HA. The use of a novel electronic nose to determine the etiology of intrapulmonary infection [Abstract]. *Anesthesiology* 1997;87:A269.
6. Thaler ER, Bruney FC, Kennedy DW, Hanson CW. Use of an electronic nose to distinguish cerebrospinal fluid from serum. *Arch Otolaryngol Head Neck Surg* 2000;126:71–74.
7. Wold S, Ebensen K, et al. Principal component analysis. *Chemometrics and Intelligent Laboratory Systems* 1987;2: 37–52.
8. De Maesschalck R. The Mahalanobis distance. *Chemometrics and Intelligent Laboratory Systems* 2000;50:1–18.