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REVIEW

The relationship between oral malodor and volatile sulfur compound–producing bacteria

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Halitosis can be a crippling social problem, and standard dental treatments and mouthwashes often provide only temporary relief. The mouth is home to hundreds of bacterial species that produce several fetid substances as a result of protein degradation. Volatile sulfur compound (VSC)-producing bacteria colonizing the lingual dorsum have recently been implicated in the generation of halitosis. Detection of VSCs, such as methylmercaptan and hydrogen sulfite, via organoleptic and objective methods, can aid in the identification of their source. Following comprehensive evaluation for possible causes, most halitosis in patients seen in an ENT practice can be localized to the tongue. We review methods of diagnosis and treatment of oral malodor from the overgrowth of proteolytic, anaerobic, gram-negative bacteria on the crevices of the lingual dorsum. Bacteriologic analysis of biofilm and scraped specimens obtained from the lingual dorsum and other oral sites, primarily gingival pockets and tonsillar crypts, can identify VSC-producing bacteria. *Porphyromonas*, *Prevotella*, *Actinobacillus*, and *Fusobacterium* species are the most common organisms identified. Halitosis is an oral phenomenon, with almost no cases originating distal to the tonsils. Halitosis arising from the lingual dorsum secondary to overpopulated VSC-producing bacteria can be successfully managed with a combination of mechanical cleansing using tongue brushes or scrapes and chemical solutions containing essential oils, zinc chloride, and cetylpyridinium chloride.

Epidemiologic studies suggest the prevalence of objectionable halitosis to be 2.4% of the adult population. According to the National Institute of Dental Research, approximately 65 million Americans suffer from halitosis at some point in their lives.[1]

Bad breath can be a crippling social problem, and standard dental treatments and mouthwashes often recommended provide only temporary relief. Halitosis differs from the temporary mouth odors caused by certain foods or drinks: persistent malodor is primarily the result of microbial metabolism. The mouth is home to hundreds of bacterial species that, as a result of protein digestion, produce several fetid substances. The role of volatile sulfur compound (VSC)-producing bacteria colonizing the tongue has been implicated as a main cause for halitosis. By detecting VSCs, such as methylmercaptan and hydrogen sulfite, with organoleptic and objective methods, one can often identify and localize their source.[1]

The goal of this review article is to provide a rational, effective approach to the care of the patient with oral malodor. Following comprehensive evaluation for possible causes, the dorsal aspect of the base of the tongue was implicated as the origin of malodor in most patients seen in an ENT practice. Oral malodor from the overgrowth of proteolytic, anaerobic gram-negative bacteria on the crevices of the lingual dorsum can be successfully diagnosed and treated.[2]

HISTORY

Bad breath has been with us for thousands of years. The problem is discussed at length in the Jewish Talmud and by Greek and Roman writers.[3,4] Islam also stresses fresh breath in the context of good oral hygiene. Ancient folk remedies abound, some of which are still in use. The Book of Genesis (Chapter 37) mentions Ladanum (mastic), a resin derived from the *Pistacia lentiscus*, a tree that has been used in Med-

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iterranean countries for breath freshening for thousands of years. Other folk cures include parsley (Italy), cloves (Iraq), guava peels (Thailand), and eggshells (China).[5,6] Experimental research on the subject dates back over sixty years.[7,8] In 1970, Tonzetich and coworkers established that oral malodor is associated with the presence of volatile sulfur compounds, primarily hydrogen sulfide and methylmercaptan.[9,10]

THE ROLE OF THE ORAL CAVITY

In most cases (up to 85%), bad breath comes from the mouth itself.[3,6,8-10] The simplest way to distinguish oral from nonoral etiologies is to compare the smell coming from the patient's mouth with that exiting the nose.[7] In people with rigorous oral hygiene, good dentition, and a healthy periodontium, the main cause of bad breath is likely to be the back of the tongue.[7,10] During evaluation the posterior lingual dorsum is most readily accessed by a gentle but thorough scraping using a disposable plastic spoon or wooden tongue blade.[10] In many cases, a yellow mucoid discharge is collected on the spoon from tongue coating. The spoon should be smelled and its odor compared to the overall mouth odor.

Although there is no direct evidence, the origin of this discharge is thought to arise from the back of the nose.[10] Postnasal drip is extremely common in halitosis and may not be indicative of any frank nasal infection or other nasal pathology. Although postnasal drip might not smell initially, its subsequent putrefaction by abundant flora at the posterior tongue renders it malodorous.

Individual lingual topography can affect the amount of coating that accumulates. People whose tongues are deeply grooved or furrowed have more potential for accumulation than those with smoother tongue surfaces. It only takes a coating of 0.1 to 0.2 millimeters to provide an environment depleted of oxygen, allowing the bacteria that cause bad breath to flourish.

There is a correlation between the amount of coating on a person's tongue and the total number of anaerobic bacteria present therein. In addition, as the anaerobic bacterial count on a person's tongue is reduced, there is usually an improvement in the odor emanating from their mouth.

In subjects with periodontal disease, the tongue has been reported to be even more malodorous.[11] Furthermore, the presence on the lingual dorsum of three specific potential periodontopathogens, *Treponema denticola*, *Porphyromonas gingivalis*, and *Bacteroides forsythus*, was found to be significantly associated with the intensity of mouth odor in two separate studies.[12,13]

BACTERIOLOGY

As with other body odors, bad breath is usually perpetrated by bacteria. Putrefaction is thought to occur under anaerobic conditions, involving a range of gram-negative microorganisms, including species like *Fusobacterium*, *Haemophilus*, *Veillonella*, *Treponema*, and *Porphyromonas* species.[14,15]

About 75 to 100 separate species of microbes live in an individual's mouth, of a total of about 700 that collectively populate all human mouths. Essentially any oral site in which microbial accumulation and putrefaction can occur may be an origin for oral malodor. In addition to the most common intraoral sites of malodor production (the tongue, interdental, and subgingival areas), other foci may include faulty dental restoration sites, sites of food impaction, and abscesses. Caries are not considered to be particularly malodorous, unless sufficiently large to allow entrapment of food particles.[16]

Dentures are another important cause of oral malodor, particularly if they are worn overnight. Usually the odor has a somewhat sweet but unpleasant character and is readily identifiable, particularly if the dentures are placed in a plastic bag and smelled after several minutes.[10]

In traditional thought, saliva plays a significant role in bad breath elimination. Bad breath levels during the day are inversely related to salivary flow.[17,18] Salivary flow is lowest overnight, due to fasting and insufficient water intake, leading to an increase in halitosis intensity. Conversely, mastication increases saliva flow, with concomitant cleansing of the oral cavity and reduction in malodor.[9,16,18] Despite these observations, the data from two clinical studies did not support any association between saliva flow rate and malodor levels.[20] The most common bacteria found among people with fresh breath was *Streptococcus salivarius*. This bacteria was present in only 1 out of 6 people with halitosis, at extremely low levels.[20]

When doubt exists concerning the oral etiology of the odor, patients may be instructed to rinse and gargle with a potent antibacterial mouth rinse, such as chlorhexidine. This has been shown in several studies to reduce odor levels significantly ($P < 0.001$).[19-21]

ODORS ORIGINATING OUTSIDE THE MOUTH

Among nonoral etiologies of bad breath, the nasal passages predominate. In such cases, the telltale odor can be smelled most strongly from the nose, rather than the mouth. Nasal odor may be indicative of either nasal infection or a problem affecting airflow associated with thick mucous secretions. Typical nasal malodor (rhinohalitosis) usually has a slightly cheesy character and differs appreciably from other types of bad breath.[10] Patients with excessive postnasal drip are more prone to bad breath. The bacteria use the mucus as a source of food, from which they are able to extract sulfur compounds. The anaerobic bacteria that cause halitosis avidly utilize cysteine and methionine from proteins found in the mucus. As a compounding factor, persons with sinus conditions often breathe through their mouths secondary to nasal congestion. The drying effect of mouth-breathing creates an environment that promotes bad breath. This may be further compounded by the anticholinergic effect of antihistamines many sinus sufferers take.

The role of the tonsils in chronic bad breath is not at all clear. The appearance of a transient odor during tonsil infections in adults and children is common. Although they may appear normal upon visual examination, when tonsils are pressed with a tongue blade they may emit foul-smelling exudates.[22,23] Dilated and deep tonsillar crypts may also contain tonsilloliths—soft, fetid stones, usually several millimeters in diameter, rough-edged, and white or yellowish in color—which themselves have a foul odor, particularly when pressed.[10]

Many nonoral systemic conditions, such as bronchial and lung infections, kidney failure, various carcinomas, metabolic dysfunctions, and biochemical disorders, can result in bad breath, but all these taken together account for only a very small percentage of those suffering from halitosis. Acetone breath was once considered a diagnostic indication for uncontrolled diabetes, but very few cases are currently encountered. A foul, fishy odor may indicate trimethylaminuria.[24] Contrary to popular conception, bad breath from the gastrointestinal tract is extremely rare.[7,8,10,16,25] The esophagus is normally collapsed and closed, and whereas the occasional belch may carry some odor up from the stomach, the possibility of gastric air reflux continuously escaping from the mouth and nose is very remote. In general, systemic disorders accounting for oral malodor amount to only 1% to 2% of halitosis clinic visits.

DIAGNOSTIC METHODS

Oral malodor may be measured in a number of ways, including studies quantitating organoleptic intensity, volatile compounds, or bacterial enzymes. Organoleptic measurements—or the use of one's nose to smell and rank the intensity of odors—are considered the gold-standard measurement of malodor. However, these have the drawback of subjectivity and discomfort for both the examiner and the test subject. Two separate judges evaluate the degree of halitosis, the intensity of which is ranked according to the Rosenberg scale (Table 1).

Table 1
Organoleptic intensity scale (based on Rosenberg)

Rating	Odor intensity
0	Odor cannot be detected
1	Questionable malodor, barely detectable
2	Slight malodor, exceeds the threshold of malodor recognition
3	Malodor is definitely detected
4	Strong malodor
5	Very strong malodor

INSTRUMENTAL ANALYSIS

The level of intraoral VSC may be estimated chairside, using portable sulfide monitors such as the Halimeter (Interscan Corp, Chatsworth, CA).[16] Sensors for VSCs have also been incorporated into probes and paddles, which can be placed directly on the tongue for measurement (Diamond Probe, Ann Arbor, MI). Other recent advances in the field include the zinc oxide sensor (Electronic Nose, National Aeronautics and Space Administration, Washington, DC), which can provide an objective quantification of halitosis. Several studies have shown that sulfide monitor readings are sensitive to reductions in mouth odor levels following the use of efficacious mouth rinses.[18-20] Despite the potential subjectivity, sulfide monitor results and odor judge scores are significantly correlated and relatively reproducible.[3] Incorporation of additional tests may further improve the association between sulfide monitor levels and odor judge scores. BANA (benzoyl-dl-arginine-α-naphthylamide) is a synthetic trypsin substrate that is converted into a colored compound when exposed to VSC-producing bacteria. In one investigation, high BANA scores were associated with the odor obtained following proximal dental floss passage and were reduced following chlorhexidine rinses.[20]

In an influential study published in 1967, Tonzetich and coworkers[26] argued that bad breath derives exclusively from sulfur-containing volatiles. They based this on the observation that other volatiles tested did not escape from the saliva into the air. However, Kleinberg[14] has recently shown that when the skin decays, non-sulfur-containing gases, such as cadaverine, putrescine, skatole, indole, butyric acid, and isovaleric acid, can be released over time. Similarly, the malodor of an extract of putrefied saliva, placed on the skin, lingers for over 2 hours. The implication is that when saliva dries out on oral surfaces, a range of VSC and other volatiles (see Table 2) are released. This is in agreement with the observation that bad breath increases when the mouth is dry.[17,18]

Despite the advantages of the tests mentioned above, clinicians interested in diagnosing bad breath must still rely on their noses to distinguish the main types of oral odors. These include 1) periodontal-type odor, which usually comes from periodontal pockets and interdental spaces, 2) odor from the posterior lingual dorsum, 3) denture odor, and 4) the characteristic nasal odor. With practice and experience, these odors become distinct and recognizable, even when found in various combinations.

In some cases, although little odor appears on the breath when the patient breathes out through the mouth, the odor becomes apparent when the patient starts speaking. Thus, in addition to asking the subject to breathe out through mouth and nose, we now routinely ask the patient to count out loud to 20, and smell the odor while the patient counts. Another

Table 2
Volatile organic compounds in the oral cavity

1) Sulfur compounds
   a) Hydrogen sulfide - H_2S
   b) Methylmercaptan CH_3SH
   c) Methanthiol
   d) Allyl mercaptan
   e) Dimethyl sulfide
   f) Dimethyl disulfide
   g) Dimethyl trisulfide
2) Short-chain fatty acids
   a) Propionic acid
   b) Butyric acid
   c) Valeric acid
   d Isocaproic acid
   e) Capric acid
   f) 2- and 3- ethyl butyric acid
   g) Lauric acid
   h) Myristic acid
3) Polyamines
   a) Cadaverine
   b) Putrescine
4) Alcohols
   a) 1-peopoxy-2-propanol
5) Phenyl compounds
   a) Indole
   b) Skatole
   c) Pyridine
6) Alkanines
   a) 2-methyl-propane
7) Ketones
8) Nitrogen-containing compounds
   a) Urea
   b) Ammonia

method is to allow the patient to exhale, to say "Ha, Ha," or to blow into a glass pipette toward the organoleptic judge.

BACTERIOLOGIC ANALYSIS

Given the multiple potential sites of malodor production, a complete head and neck examination, including nasal endoscopy, flexible laryngoscopy, and site-directed cultures is indicated, as is measurement and quantification of malodor. Bacteriologic analysis of both the biofilm and scraped specimens obtained from the lingual dorsum and other oral sites (primarily tonsil crypts and gingival pockets) can identify the VSC-producing bacteria. *Porphyromonas*, *Prevotella*, *Actinobacillus*, and *Fusobacterium* species are the most common organisms identified from cultures of the tongue. In the teeth, odor-causing bacteria include *T. denticola*, *P. gingivalis*, and *B. forsythus* (see Table 3).

Detection of pathogens is traditionally performed by culture. Since there is an inherent bias against the detection of microaerophilic and anaerobic organisms by traditional culture techniques, polymerase chain reaction (PCR) has become the favored detection modality.

PCR is rapid, inexpensive, and simple and can produce relatively large numbers of DNA copies, even if the source DNA is of relatively poor quality (eg, from saliva or the tongue coating). PCR methodologies have been optimized for quick and accurate determination of bacterial gene expression from different sites in the oral cavity. Recent studies using real-time PCR provided quantitative analysis of 5 common bacteria responsible for oral malodor in saliva and lingual dorsum, namely *Porphyromonas gingivalis*, *Tanerella forsythia*, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Treponema denticola*. These results suggest PCR may be a helpful tool for analyzing the relationship between oral bacteria and halitosis, and for monitoring the effectiveness of various therapeutic modalities.[27,28]

TREATING BAD BREATH

The best way to treat bad breath is to instill in patients good oral hygiene practices (Table 4).[9,16] Although patients often balk at using dental floss, once the connection is made between flossing and fresh breath (eg, by simply asking the patients to smell their own floss following each passage), compliance improves.[16] In one study, subjects who flossed were found to have significantly less mouth odor (P = 0.016), salivary odor ($P < 0.001$), and salivary cadaverine levels (P = 0.011) than those who did not. Furthermore, 1 year following the initial oral malodor examination, the percentage of subjects who flossed their teeth rose from

Table 3
Bacteriology of the oral cavity in halitosis with regard to VSC production

H_2S from cysteine	CH_3SH from methionine	H_2S from serum	CH_3SH from serum
Peptosteptococcus anaerobius	Fusobacterium nucleatum	Prevotella intermedia	Porphyromonas gingivalis
Micros prevotti	Fusobacterium periodonticum	Prevotella loescheii	Treponema denticola
Eubacterium limosum	Eubacterium spp.	Porphyromonas gingivalis	Porphyromonas endodontalis
Bacteroides spp.	Bacteroides spp.	Treponema denticola	
Centipedia periodontii			
Selenomonas artermidis			

Table 4
Several of the central concepts in diagnosis and treatment of oral malodor

Bad breath is a common condition, usually comes from the mouth itself, and rarely from the gastrointestinal tract or from a systemic disorder.
The dentist/physician has the primary responsibility for diagnosing and treating bad breath.
The posterior tongue dorsum is the most frequently overlooked source of oral malodor. It can be readily sampled using a plastic spoon.
Patients complaining of bad breath should be assigned a separate appointment, and should be encouraged to bring along a family member or close friend (confidant).
Because of the difficulty inherent in smelling our own bad breath, many individuals harbor grossly exaggerated concerns while others remain unaware that they suffer from the problem.
Although correlative quantitative measurement techniques are available and helpful, the clinician should also make a differential judgment based on actual smelling of the odor emanating from the patient's mouth and nose.
In most cases, bad breath can be ameliorated by adequate hydration, proper dental care, oral hygiene, deep-tongue cleaning, and, if necessary, rinsing with an effective mouthwash.

31% to 65%.[17] Other interdental cleaners (such as anatomic plastic toothpicks) can also be effective in identifying and cleaning sites of odor production.

Mechanical reduction of malodor and of the intraoral bacterial count may be achieved by disrupting the tongue biofilm, thus decreasing the production of VSCs and other volatile organic compounds (VOCs). Common methods include tongue brushing, tongue scraping, and chewing gum. Gentle but effective deep-tongue cleaning should be an important daily routine. A variety of tongue brushes and scrapers have been produced in recent years. The tongue should be brushed in a gentle but thorough manner, in posterior to anterior direction, keeping in mind that the posterior portion is the area that is the least accessible but that smells the worst.[7,8,10] Even patients with a significant gag reflex can get used to cleaning the back of their tongue with some practice. Because bad breath is worse when the mouth dries out (eg, at night, while fasting), subjects should also be encouraged to maintain a good hydration.[16]

Even with the implementation of good oral hygiene, many patients continue to have halitosis of oral origin. In such instances, rinsing and gargling with an efficacious mouthwash may be advised. It should be kept in mind, however, that many mouthwashes contain components that may have a nonbeneficial effect on oral soft tissue (eg, alcohol, sodium dodecyl sulfate, or strong oxidizing agents). Optimally, oral mouthwashes are used before bed, since the residue of the mouth rinse may thus remain in the oral cavity for a longer period of time, to greater effect. In addition, bacterial activity leading to bad breath is greatest at night.[9]

In addition to these methods, reduction of oral malodor may be aided by the use of active chemical agents, including the delivery of active antimicrobial compounds via mouth rinses, dentifrices, or lozenges. The various compounds studied are listed in Table 5. These compounds decrease the bacterial load and thus decrease the VSC and VOC production. Other methods to reduce halitosis include changes in diet (high-protein diets tend to increase VSC and VOC production) and prevention of oral mucosal dehydration by oral intake and stimulation of salivary flow (lysozymes in saliva inhibit bacterial growth). Of note, although there may be a dramatic reduction in an individual's bad breath following consultation and treatment, the patient may find it difficult to sense the improvement. This problem can be addressed with the help of a confidant, who can monitor changes over time.

Any chemical agent or mechanical means of reduction of halitosis should meet the following criteria:

1) Safe for long-term use (defined as greater than 6 months)
   a) Without causing an overgrowth of opportunistic and pathogenic microorganisms (eg, *S. aureus*, *Pseudomonas*, *E. coli*, yeasts, etc.), and
   b) Without damage to soft or hard tissues of the oral cavity

2) Reduction of halitosis to an average organoleptic intensity rating of 2 ± 0.5 (mild malodor) on the Rosenberg intensity scale.

The future of oral malodor diagnosis and treatment includes the production of an electronic nose for quantification of all VOCs, not VSCs alone, to quantify malodor-causing compounds properly. The use of ultrasonic disrupters of biofilm, laser-like light devices, and lasers themselves holds good promise for treatment, either in isolation or in conjunction with photoactive chemical compounds.[29]

Table 5
Studied antimicrobial compounds and delivery method for reduction of oral malodor

Compound	Delivery
Triclosan	Dentifrice
Tin fluoride, sodium fluoride, and triclosan	Dentifrice
20% to 65% bicarbonate and Zn salts	Dentifrice
Hydrogen peroxide	Dentifrice
Bicarbonate	Dentifrice
Zinc chloride	Mouth rinse
Listerine	Mouth rinse
Chlorine dioxide	Mouth rinse
Cetylpyridinium chloride	Mouth rinse
Chlorhexidine	Mouth rinse

CONCLUSIONS

Halitosis is an oral phenomenon, with few cases originating distal to the tonsils. Halitosis arising from the lingual dorsum from overpopulated, VSC-producing bacteria can be successfully managed with combinations of mechanical and chemical cleansing.

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