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Research Article

The relationship between bad breath of the oral cavity with bacterial infection from smoking

Susan F.K. Al-Sudani*, Zainab Tariq Al-Atya

Al-Bayan University/ College of Dentistry
*Email: Susan.f@albayan.edu.iq

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Abstract

This study included 72 participants with oral halitosis (35 men and 37 women), who had been clinically diagnosed and ranged in age from 14 to 70. 95 samples were obtained using sterile paper points (size 30) or sterile cotton swabs, in either aerobic or anaerobic conditions, thioglycolate broth was transported and blood agar was used for culture (for 48–72 hours). The bacteria were recognized by means of biochemical testing, tests for antibiotic susceptibility, and morphological and cultural features. Results revealed that 13 different bacterial species were isolated in this study, including Bacteriodes spp. (26 isolates), Viridans Streptococci (18 isolates), Peptostreptococci (19 isolates), Actinomyces spp. (15 isolates), Porphyromonas spp. (8 isolates), Fusobacterium spp. (9 isolates), Veillonella spp. (5 isolates), Streptococcus spp. (10 (2 isolates). The dominant anaerobic bacterial species and genera in instances of halitosis is the conclusion.

Keywords: Halitosis, malodor, anaerobic bacteria, aerobic bacteria, smoking, oral cavity

Introduction

Halitosis, sometimes referred to as bad breath, breath malodor, and oral malodor, is a prevalent illness that affects 15–60% of persons in the world, with notable geographical differences (Seemann et al., 2016; Alsadhan et al.,2016). An offensive odor in exhaled breath is a symptom of halitosis. (Al-Humaidi et al., 2017). Poor dental hygiene, periodontal conditions, dry mouth, tobacco use, alcohol usage, eating habits, diabetes, and obesity are just a few of the many factors that contribute to halitosis. Other factors include becoming older, stress, poor body hygiene, and the use of specific drugs (Wu et al., 2020; Mortazavi et al., 2020).

Halitosis comes in two different forms: extraoral (EOH) and intraoral (IOH). (Miyazaki, 1999). Only

a tiny fraction (5–10%) of instances of halitosis have extraoral causes. This kind of halitosis may result from diabetes, metabolic conditions, kidney and liver problems, as well as from specific medications and meals (Tangerman and Winkel, 2010). However, the majority of halitosis cases (80–90%) begin in the mouth, and this type is frequently brought on by poor oral hygiene, dental plaque, dental caries, gingivitis, stomatitis, periodontitis, tongue coating, and, in uncommon cases, oral cancer. (Renvert et al., 2020). Xerostomia, or dry mouth, may also contribute to bad breath (Porter et al., 2004), however a connection hasn't always been found. (Porter, 1999). The coating of the tongue, which accounts for 43.4% of cases of IOH in healthy subjects, is the most frequent source of oral odor (Quirynen et al., 2009). The architectural structure of the tongue facilitates the accumulation of bacteria, a significant number of desquamated epithelial cells, blood metabolites, food debris, and leukocytes from periodontal pockets on the tongue's surface. The dorsoposterior region of the tongue is the main source of oral odor since this region is where the majority of the anaerobic bacteria that cause odor proliferate (Allaker et al., 2008).

IOH is produced as a result of the metabolism of bacteria in the oral cavity, which mostly produces volatile organic compounds (VOCs). Over 700 chemicals have been found in the oral cavity, but because to their high volatility and low odor threshold, volatile sulfuric compounds (VSC), which include hydrogen sulfide and methyl mercaptan, are the most prevalent (90%) in IOH (Laleman et al., 2020). Dimethyl sulfide is a highly common VSC in halitosis as well, however it is connected to EOH. (Van Den Velde et al., 2009). The primary substances that cause oral malodor are these VSCs. (Saad et al.,2018). Short-chain fatty acids, amines, and indols are the following groups of VOCs that also contribute to bad breath. The amount of volatiles generated is connected with the density of the anaerobic bacteria present in the tongue coating, which metabolize sulfur amino acids primarily to form VSCs (Mostefa-Saad et al., 2006). There is evidence that VSCs and other VOCs harm oral soft tissues and cause alterations that could result in carcinogenesis. (Karpinski et al., 2019). Diamines, such as trimethylamine, putrescine, and cadaverine, which are created when bacteria putrefy food, are other substances that cause halitosis (Dadamio et al., 2011).

To date, there hasn't been a methodical investigation into how nicotine delivery systems, including cigarettes, affect halitosis. Using cutting-edge omics technologies for breath and microbiome investigations, we will go over the pertinent data in this paper and put up a methodology for methodically comparing and contrasting the impacts of cigarette smoke and other nicotine delivery systems on IOH. Although it is generally agreed that Gram-negative oral microorganisms are to blame for the majority of cases of oral malodor, the precise species that produce the foul odor is unknown, anaerobic bacteria are to blame. Therefore, it seems that oral malodor is most likely caused by the

mixed microbiota's metabolic activity rather than the number of bacteria present or the genus types (Wray *et al.*, 2003).

Materials and methods

The study involved 72 adult patients (35 men and 37 women), ranging in age from 14 to 70, who were patients of the dental educational hospital, oral diagnosis division, and college of dentistry at Albayan University. Halitosis was a problem for each and every patient.

Patients who had taken antibiotics within the preceding two weeks, had odor-producing foods the day before the test or the morning of it, smoked within an hour of the test, chewed gum, used scented personal care items, or cleaned or rinsed their teeth with strong odorous substances were eliminated. on the oral diagnosis sector, a dental examination was carried out on a dentist chair with artificial lighting. The same examiner used and gathered various clinical characteristics.

After a thorough clinical examination, the examiner had to look for the causes of the halitosis, which included deep pockets, heavy calculus, a huge destructive carious tooth, and a retained root. After cotton rolls were used to isolate the bacteria, a single sterile point size 30 (Vevey Suisse, Switzerland) was inserted into the prospected area for 40 seconds, and then it was immediately placed in sterile screw-capped vials containing 4 milliliters of thioglycolate broth, which serves as a reducing transport medium for anaerobic bacteria. A sterilized tongue scraper had to be used to repeatedly scrape the tongue. (Trisa, Swizerland). A viscous brown fluid is produced by this scraping. Another sterile point size 30 was put into the brown fluids using sterile tweezers for 40 seconds, and it was then immediately placed into another sterile screw-capped vial containing four milliliters of thioglycolate broth. As a result, even though we had four vials for each patient, only one included tongue scraping samples in cases with adequate oral hygiene (healthy gingivae and sound teeth). Each screw-capped vial of thioglycolate broth was infected in a sterile environment using a sterile cotton swab before being streaked over two newly prepared blood agar plates. (one for aerobic and another for anaerobic). For 48–72 hours at 37 C, blood agar plates were incubated anaerobically using an anaerobic jar with gas generating equipment. The other blood agar plates were incubated for 48 to 72 hours at 37 C in the same incubator. (Summanen, 1999). Utilizing a variety of techniques, colonies with distinct traits were isolated and identified. The Bergey's manual of determinative bacteriology was used for identification in this study.(Forbes et al., 2007; Al-Ubaidy and Shareef, 2008). A crucial quick tool for diagnostics is gram stain. Rapid presumptive identification of some anaerobic bacteria can be accomplished by detecting fluorescence under long wave UV light (360 nm). The test for detecting capsules was carried out against Vancomycin, Kanamycin, Colistin, Optochin, Metronidazole, and

Penicillin. It was also used to ensure that the VITEK_2 system, which was also used to identify by sensitive tests of antibiotics recommended by the World Health Organization (WHO), was used.

Results

The seventy-two participants in the study's samples all had oral halitosis. They sought a diagnosis and treatment at the College of Dentistry at Albayan University. The sample was made up of 37 females and 35 guys, whose ages ranged from 14 to 70.
Tables 1 and 2 show the results of the VITEK-2 sensitivity test on the 126 bacterial samples (from 13 distinct bacterial species) taken from 72 patients who had halitosis.

Table 1. The relationships between Age , gender and Halitosis related with smoking

	No-Halitosis (*n* = 40)	**Halitosis (*n* = 32)**	***p*-Value ***	**Total (*n* = 72)**
Age (years), mean (±SD)	**59.5 (±3.6)**	**49.9 (±3.2)**	**0.066**	**54.6 (±17.6)**
Gender *n* (%)				
Male	**20 (50.0)**	**15 (46.9)**	**0.817**	**35 (48.6)**
Female	**20 (50.0)**	**17 (53.1)**		**37 (51.4)**
Smoking habits, *n* (%)				
Current smoker	**13 (32.5)**	**7 (21.9)**	**0.429**	**20 (27.8)**
Non-smoker	**27 (67.5)**	**25 (78.1)**		**52 (72.2)**

Table 2. Microscopical and Macroscopical Characteristics of the Isolated Bacteria

	Organism	**No. of isolates**
1	*Bacteriodes species*	26
2	Viridans Streptococci	**18**
3	*Peptostreptococci* species	19
4	*Actinomyces species*	15
5	*Porphyromonas* species	8
6	*Fusobacterium* speices	9
7	*Veillonella* species	**5**

8	*Streptococcus spp.*	10
9	*Prevotella* species	2
10	*Propinobacterium* Species	3
11	*Tetragenococci* Species	5
12	*Eubacterium* species	4
13	*Staphylococcus aureus*	2
	Total	126

Table 3. Antibiotic sensitivity tests for identification of isolated bacteria

	Organism	**Vancomycin 30 µg**	**Kanamycin 30 µg**	**Colistin 10 µg**	**Optochin 30 µg**	**Metronidazole 5µg**	**Penicillin 10 µg**
1	*Bacteriodes species*	R	R	R	……	S	R
2	Viridans Streptococci	…..	R	…..	R	…..	S
3	*Peptostreptococci species*	S	S	R	…..	…..	…..
4	*Actinomyces species*̧	R	S	R	…..	R	……
5	*Porphyromonos species*	S	R	R	…..	S	…..
6	*Fusobacterium speices*	R	S	S	……	S	…..
7	*Veillonella species*	*R*	*S*	*S*	….	….	….
8	*Streptococcus Species*	*S*	*S*	*R*	*R*	*R*	*R*
9	*Prevotella species*	*R*	*R*	*S*	…..	*S*	…..
10	*Propinobacterium*	*R*	*R*	*S*	*R*	*R*	*S*

	Species						
11	*Tetragenococci Species*	*S*	.....	.....	*S*	*R*	*S*
12	*Eubacterium species*	.....	*S*	*R*	....	....	*R*
13	*Staphylococcus aureus*	*R*	*R*	*R*	*R*	*R*	*R*

S=microorganism was sensitive to the antibiotic, R=microorganism was resistant to the antibiotic

…. =the antibiotic test was not necessary

Discussion

Standard dental procedures and mouthwashes, which are frequently advised, only offer brief relief from bad breath, which can be a crushing social issue. Halitosis is distinct from the transient mouth scents brought on by particular meals or beverages because it is predominantly brought on by microbial metabolism. (Zanetti et al., 2021).

Only the colon in humans has a higher density and greater variety of bacteria than the mouth. (Mok et al., 2017). Around 700 species of bacteria are thought to make up the majority of the bacteria in the mouth cavity.(Krishnan et al., 2017). The development of IOH is primarily influenced by the tongue's microbiota. (Seerangaiyan et al., 2017). The papillae, deep fissures, and crypts found in the tongue structure create a low oxygen potential environment that is perfect for the growth of anaerobic bacteria linked to halitosis. (Seerangaiyan et al., 2018). Patients with halitosis appear to have more diverse oral bacterial populations than healthy individuals. (De Geest et al., 2016); IOH is linked to an increase in the activity or abundance of specific bacterial taxa in tongue biofilms, including Fusobacterium, Porphyromonas, Prevotella, and Tannerella. (Ren et al., 2016). Halitosis is thought to be caused by a particular biofilm on the dorsal surface of the tongue: A large portion of the bacterial biofilm in persons with halitosis is made up of Fusobacterium nucleatum and Streptococcus spp., according to a study that combined confocal laser microscopy and fluorescence in situ hybridization examinations. (Bernardi et al.,2019). This study also found that Salmonella infantis, Streptococcus mitis/oralis, Streptococcus pseudopneumoniae, and Prevotella spp. were frequently found on the tongue coverings of people with halitosis. Another study that used a broad methodology found a connection between Actinomyces graevenitzii and Veillonella rogosae and the onset of IOH (Bernardi et al. 2019). The density of the bacterial population on the tongue appears to be related to the creation of VSCs, rather than the biofilm's thickness, which does not appear to be related (Bernardi et al., 2020).

Halitosis is linked to the presence of gram-negative bacteria. (Veloso et al.,2020). Prevotella, Alloprevotella, Leptotrichia, and Peptostreptococcus are more prevalent in halitosis patients' bodies than in healthy ones (Ye et al.,2019). Periodontal problems are linked to red complex members Tannerella forsythia, Porphyromonas gingivalis, and Treponema denticola, which also have a positive effect on IOH. Gram-negative anaerobes are the most active hydrogen sulfide makers. (Silva et al., 2017). It has been demonstrated, for instance, that areas of periodontal inflammation produce large amounts of hydrogen sulfide and methyl mercaptan. (Silva et al., 2020). Additionally, the species of Porphyromonas, Prevotella, and Treponema denticola may be crucial in giving amino acids to other anaerobic bacteria so they can produce methyl mercaptan and hydrogen sulfide (Suzuki et al., 2019). Porphyromonas gingivalis, Prevotella intermedia, and Fusobacterium nucleatum are primarily responsible for the production of indole and skatole (also known as 3-methylindole). Codipilly and Kleinberg (2008). Furthermore, gram-positive bacteria aid gram-negative anaerobic bacteria in IOH by eliminating the sugar chains from glycoproteins and supplying the required proteins during the proteolytic processes. (Suzuki et al., 2019).

The oral microbiome's composition is significantly influenced by CS. The connection between CS, microbiota, and IOH will be discussed in the section after this one. The tongue is a significant source of oral malodor creation, even if periodontal disease and other causes seem to make up a very tiny fraction of the overall problem. The papillary form of the dorsum creates a unique biological niche in the oral cavity by offering a sizeable surface area that promotes the accumulation of oral debris and bacteria. The bacterial makeup of the tongue is still little understood, and because of its complexity, the characteristics of tongue have not been widely studied, despite the fact that the bacteria of the tongue have been identified as a major source of odor production in people with halitosis. (Cortelli *et al*., 2008).

Since the majority of the microorganisms causing oral halitosis are non-cultivable, we advise employing Polymerization Chain Reactions (PCR) to identify them.

Conclusions

A significant risk factor for halitosis is CS. Alternative nicotine-delivery systems, such as EVPs and HTPs, may lessen the health concerns connected with CS for smokers who are unable to quit. So far, very few systematic studies have looked at how CS affects halitosis, and none have looked at how HTPs and EVPs affect it. Self-assessment studies have a lot of drawbacks because the participants' opinions aren't trustworthy. This forces the scientific community to create a plan for an accurate comparison of these new goods to smoke. Using such a method, we may evaluate the effects of various nicotine delivery methods on oral bacteria and compare how they affect halitosis. In comparison to

cigarette smoke, the usage of alternate nicotine delivery products has the potential to have a greater influence on halitosis, which our suggested methodology can the ability quantify and mechanistically address. The outcomes of such a thorough investigation could be used to develop treatments that lessen the potential negative effects on the breath odour of substitute nicotine delivery systems. The suggested approach will be a crucial step in further identifying the hazards and safety of using these items for consumers.

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