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The Occurrence of Microorganisms on the Root of the Tongue in Patients with Halitosis

Występowanie drobnoustrojów na nasadzie języka u chorych z halitozą

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Abstract

Background. The halitosis (bad breath) is a common phenomenon, usually the result of bacterial metabolism in the oral cavity. The production of volatile sulfur-containing compounds (vsc) in the mouth, in particular hydrogen sulfide and methyl mercaptane, is the main source of the malodor.
Objectives. Determination of the differences in microbial flora colonizing the root of the tongue in patients with halitosis and healthy people.
Material and Methods. The samples for microbial culture were taken from the root of the tongue of 65 patients with halitosis and 20 healthy people (the control group).
Results. In patients samples various aerobes were cultured (20% of total bacteria) compared to 4.4% in control group. Also the big diversity in the anaerobes from malodor samples were noticed. The producing of vsc depends probably of total composition and proportion in mouth microflora, not only particular strains (**Adv Clin Exp Med 2005, 14, 4, 771–775**).

Key words: halitosis, bacterial flora.

Streszczenie

Wprowadzenie. Halitoza (nieświeży oddech) jest zjawiskiem powszechnym, najczęściej rezultatem aktywności metabolicznej flory jamy ustnej. Produkcja związków lotnych zawierających siarkę (v.s.c.) w ustach, w szczególności siarkowodoru i merkaptanu, jest głównym źródłem nieświeżego oddechu.
Cel pracy. Określenie różnic we florze bakteryjnej kolonizującej nasadę języka pacjentów z halitozą i osób zdrowych.
Materiały i metody. Próbki do hodowli bakterii pobrano z nasady języka 65 pacjentów cierpiących na halitozę i 20 osób zdrowych (grupa kontrolna).
Wyniki. W wymazach od pacjentów wyhodowano różnorodne bakterie tlenowe (20% wszystkich szczepów) w porównaniu do 4,4% w grupie kontrolnej. Duże zróżnicowanie odnotowano również wśród bakterii beztlenowych. Produkcja v.s.c. prawdopodobnie zależy od całkowitego składu i proporcji flory jamy ustnej, a nie od występowania poszczególnych szczepów (**Adv Clin Exp Med 2005, 14, 4, 771–775**).

Słowa kluczowe: halitoza, flora bakteryjna.

Halitosis (oral malodor or bad breath) has a significant impact on our daily social life to those who suffer from it. Bad breath is a common phenomenon, usually the result of bacterial metabolism in the oral cavity [1, 2]. The production of volatile sulfur-containing compounds (vsc) in the mouth, in particular hydrogen sulfide and methyl mercaptane, is the main source of the malodor. The production of vsc resulting from the microbial degradation of amino acids in the diet, desquamated epithelial cells, serum and saliva [3].

The oral surface are colonized by over 500 bacterial species [4], many of which can degrade proteins, peptides and amino acids to the foul-smelling

volatile sulfur compounds, fatty acids and polyamines [5, 6]. *In vitro* many bacteria as well as oral specimens such as saliva [7], plaque [8, 9] and tongue coating, can produce the vsc. The methylmercaptan (CH_3SH) is produced by *Treponema denticola*, *Porphyromonas* sp., *Fusobacterium* sp., *Eubacterium* sp., *Bacteroides* sp., and hydrogen sulfide (H_2S) by *Peptostreptococcus* sp., *Eubacterium* sp., *Bacteroides* sp., *Selenomonas* sp., *Prevotella* sp., *Treponema denticola*, *Porphyromonas* sp. [11]. Short chain fatty acids such as butyric, propionic, valeric etc. contribute to the complex mixture of odorous molecules found in the exhaled air. *In vivo* the same bacteria would degrade the sulfur-containing peptides and amino acids, that are found in saliva, crevicular fluid, blood, food retained and desquamated epithelia [10, 11]. Each of the 500 species has evolved a nutritional strategy that allows it to persist in the crowded, competitive oral environment. Some malodorous species might produce vsc from amino acids: *Porphyromonas gingivalis*, *Peptostreptococcus anaerobius*, *Fusobacterium nucleatum* and *Treponema denticola* [5, 12]. Tongue surface is composed of blood components, nutrients, large amounts of desquamated epithelial cells and bacteria. The studies indicate that the bacteria residing on the tongue make the dominant contribution to oral malodor [13, 14]. Despite the fact that the tongue has the largest bacterial load of any oral tissue and makes the greatest contribution to the bacteria found in the saliva, very little is known about the flora indigenous to the tongue. The considerable basic microbial studies on the composition of normal flora are necessary before insights into the presence of a flora unique to malodor subject can be determined.

The main aim of the paper was to study the differences in types of microorganisms colonizing the root of the tongue in patients with halitosis and healthy people.

Material and Methods

Material of the study was of 65 patients (24 male and 41 female) with halitosis. The control group consisted of 14 healthy womean and 6 men. The age of patients ranged from 20 to 70 years. The diagnosis and treatment of the patients were carried out in the Department of Oral Pathology and Prosthodontics. The clinical examination included: plaque index acc Silness and Löe PI1, plaque index acc O'Leary PI2, API acc Lange; index of inflammation: Gingival Index PBI acc Saxer and Mühlemann; pocket depth: at 2 approximal sites (GK1) at 4 sites (Gk2), the number of pockets deeper than 5 mm (GK3).

The microbiological investigation was performed in the Bacteriology Department of Korczak Children's Hospital in Wrocław. The samples were taken from the root of the tongue of the patients on Portagerm Amies Agar swabs (bioMérieux, France) and sent immediately to Bacteriology Department. For anaerobes the Schaedler Agar + 5% SB (SheepBlood), Schaedler NeoVanco Agar + 5% SB and Columbia CNA Agar + 5% SB (bioMérieux, France) were used. The growing of anaerobic bacteria was carried out in anaerostat Genbox System (bioMérieux, France) at 35°C for 7 days. The samples for aerobic bacteria were placed on Columbia Agar + 5% SB, MacConkey Agar, Mannitol Salt Agar (Biomerieux, France) and incubated at 35°C for 24 h.

The type of bacteria was identified on the base of microscopic morphology and metabolic properties (test for catalase, coagulase, test ID32 E, ID32 GN, ID 32 Staph, ID32 A (bioMérieux, France)).

Yeast were cultured and identified on Chromagax Candida (Emapol, Poland).

Results

The physiological flora including *Streptococcus orale* and *Neisseria* sp. were cultured from 94% of patients with halitosis and from 100% of the control group, and the yeasts from 7.7% and 30% respectively.

The spectrum of bacteria isolated from the root of the tongue is placed in the Table 1.

In the tested group, 20% of total bacteria various aerobes were cultured. They belong to Gram-positive 2.1% (*Staphylococcus* sp., *Streptococcus* sp.), nonfermentative rods 6.2% (*Acinetobacter* sp., *Aeromonas* sp., *Comamonas* sp., *Pseudomonas* sp.) and *Enterobacteriaceae* 11.7% (*E.coli*, *Klebsiella* sp., *Enterobacter* sp., *Serratia* sp., etc.). To compare from the healthy patients only *Enterobacteriaceae* 4.4% were isolated. Also the big diversity in the anaerobes from malodor samples compared to control group were noticed. The most often *Prevotella denticola* (10%), *Prevotella oralis* (13.8%) and *Veillonella* sp. (20.4%) were cultured. The significant differences between the anaerobes occurrence in tested group and control group were noticed for *Eubacterium* sp. (2.8% versus 17.4%), *Leptotrichia buccalis* (6% versus 0%), *Peptostreptococcus prevoti* (3.9% versus 0%) and *Prevotella oralis* (13.8% versus 32.6%).

Discussion

The tongue is colonized immediately after birth by anaerobes such as *Prevotella* sp. and *Fusobacterium nucleatum* [15]. Their number and

Table 1. Microbial flora isolated from the root of the tongue

Tabela 1. Flora bakteryjna izolowana z nasady języka

Bacteria (Bakterie)	No. of strains isolated from patients with halitosis (Liczba szczepów wyizolowana od pacjentów chorych na halitozę) %	No. of strains isolated from control group (Liczba szczepów wyizolowana od grupy kontrolnej) %
Aerobes (Tlenowce)		
Acinetobacter lwoffii	2 (1.4)	0
Acinetobacter jonsonii	1 (0.7)	0
Aeromonas hydrophila	1 (0.7)	0
Cedecea sp.	1 (0.7)	0
Comamonas testosteroni	1 (0.7)	0
Enterobacter cloacae	4 (2.8)	0
Enterobacter amnigenus	1 (0.7)	0
Enterobacter asburiae	1 (0.7)	0
Escherichia coli	3 (2.0)	1 (2.2)
Hafnia alvei	1 (0.7)	0
Klebsiella pneumoniae	3 (2.0)	1 (2.2)
Pseudomonas fluorescens	1 (0.7)	0
Pseudomonas putida	3 (2.0)	0
Serratia liquefaciens	1 (0.7)	0
Serratia marcescens	2 (1.4)	0
Staphylococcus aureus	2 (1.4)	0
Streptococcus beta-haemolyticus	1 (0.7)	0
Anaerobes (Beztlenowce)		
Actinomyces israeli	1 (0.7)	0
Actinomyces meyeri	3 (2.0)	3 (6.5)
Actinomyces naeslundii	4 (2.8)	0
Actinomyces sp.	1 (0.7)	0
Bacteroides capillosus	2 (1.4)	0
Bifidobacterium adolescentis	4 (2.8)	0
Capnocytophaga sp.	6 (3.9)	1 (2.2)
Clostridium butyricum	0	2 (4.4)
Clostridium fallax	1 (0.7)	0
Clostridium tyrobutyricum	3 (2.0)	0
Clostridium sp.	2 (1.4)	0
Eubacterium sp.	4 (2.8)	8 (17.4)
Gemella mobillorum	1 (0.7)	0
Leptotrichia buccalis	9 (6.0)	0
Peptostreptococcus prevoti	6 (3.9)	0
Prevotella buccae	4 (2.8)	1 (2.2)
Prevotella denticola	15 (10.0)	5 (10.8)
Prevotella intermedia	1 (0.7)	0
Prevotella oralis	21 (13.8)	15 (32.6)
Propionibacterium propionicum	1 (0.7)	0
Veillonella sp.	31 (20.4)	9 (19.5)
Total (Razem)	152 (100.0)	46 (100.0)

the presence of other *Treponema denticola* and *Selenomonas salivarius* increase at the time of the primary erupion of teeth [16]. The analysis of tongue samples from healthy individuals identified high titers of aerobic *Streptococcus parasanquis* and *Streptococcus salivarius* (70% of total). In addition ten new species belonging to anaerobic genera appeared: *Bifidobacterium*, *Eubacterium*, *Leptotrichia*, *Porphyromonas* [13]. Young children with oral malodor had a significant increase in the salivary levels of *Prevotella oralis* and *Prevotella melaninogenica* compared with age-matched children without malodor [17]. This suggests that the anaerobes were contributing to the malodor. The analysis of tongue samples from clinically diagnosed malodor subjects identified high titers of *Fusobacterium periodonticum*, *Neisseria flavescens* and *Streptococcus* sp. Malodor samples contained species of *Actinomyces*, *Eubacterium*, *Leptotrichia*, *Megasphaera*, *Por-*

phyromonas, *Prevotella*, *Selenomonas*. Generally, malodor subjects had a much greater species diversity, with 50 distinct phylotypes detected compared to only 28 in the non-malodor subjects [14, 18, 19]. In malodor flora most often *Porphyromonas gingivalis*, *Fusobacterium*, *Treponema denticola*, *Peptostreptococcus* sp., *Eubacterium* sp., *Selenomonas* sp. and *Bacteroides* sp. were isolated [20–22].

In presented study, the authors obtained similar results with various types of anaerobes and aerobes isolated from malodor patients compared to healthy group. But the *Porphyromonas gingivalis*, *Fusobacterium* and *Treponema denticola* were not cultured. The significant higher frequency isolation of *Eubacterium* sp. and *Prevotella oralis* in control group was found. Low number of *Enterobacteriaceae* strains were isolated in healthy individuals compared to various aerobes from patients with halitosis. These data are similar to Goldberg paper [23] where *Klebsiella* sp. and *Enterobacter cloacae* isolated from saliva produced an odor resembling bad breath.

In conclusion, in malodor samples great species diversity of aerobes and anaerobes were found. The producing of vsc depends probably of total composition and proportion in mouth microflora, not only particular strains.

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