Microorganisms in root carious lesions in adults

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Abstract

Purpose: Root caries is emerging as a significant problem in the middle aged and elderly subjects because of the improving general health conditions, and medical and technological advances. The purpose of this investigation was to assess the prevalence of aerobic and anaerobic bacteria as well as yeasts of *Candida* genus in root carious lesions in middle-aged and older adults.

Material and methods: Specimens of root carious lesions were collected from 78 adults for bacteriological and mycological studies. Standard procedures of culture, isolation, and identification of aerobic and anaerobic bacteria, and fungi were used in the study.

Results: The analysis of results was performed independently in two age groups of adults, i.e. 52 subjects aged 35-44 years (middle age) and 26-aged 55-72 years (older age). There were 120 bacterial strains isolated from root carious lesions in middle-aged subjects, 63 (52.5%) strains belonged to 5 genera of aerobic bacteria and 57 (47.5%) – to 7 genera of anaerobic bacteria ($p>0.05$). While in the second group, 85 strains were isolated, 54 (63.5%) – 6 genera of aerobic bacteria and 31 (36.5%) – 4 genera of anaerobic bacteria ($p=0.0004$). There were no differences between the isolation rate of a various species in both examined groups, except for *Streptococcus* spp., *S. oralis*, *Micrococcus* spp., *Neisseria* spp. and *Veillonella* spp., which statistically significantly most frequent occurred in elderly ($p<0.05$). The yeasts of *Candida* genus of 4 species (*C. albicans*, *C. lusitaniae*, *C. pelliculosa*, and *C. pulcherrima*) were isolated from middle-aged subjects (32.7%) with the comparable rate to older adults (30.8%; only *C. albicans*) ($p>0.05$). Among all isolated microorganisms, *Candida* spp., were comprised about 10% in both examined groups ($p>0.05$).

Conclusions: Aerobic Gram-positive cocci (*Staphylococcus* spp. and *Streptococcus* spp.) as well as anaerobic ones (*Peptostreptococcus* spp.), and *Candida albicans* were occurred most frequently in root carious lesions in middle--aged and older adults.

Key words: root caries, middle-aged subjects, older adults, aerobic/anaerobic bacteria, *Candid*a spp.

Introduction

Root surface caries, as the name implies, occurs on root cementum or dentine and is caused by a microbial biofilm. The disease is secondary to gingival recession, since, in a healthy mouth, cementum and dentine are not exposed to the microflora and, therefore, are non expose for colonization [1-3]. Gingival recession can be caused by a number of factors, including old age (the most common factor), mechanical injury (excessive tooth brushing) or periodontal treatment regimens [3]. In developed countries proportion of the population over 65 years of age is increasing, additionally, the percentage of these remaining dentate is also increasing. A survey carried out by Steele et al. [4] in 1991-92 showed, amongs other things, that in Southern England 67% of patients over 60 years were dentate compared with equivalent cohort in 1962, where only 15% remained dentate.

The aetiology of root caries is multifactorial of which microbiological factor plays a critical role [1-3]. The microbiological nature of the associated plaque biofilm is different from that associated with crown caries (supragingival plaque) even though it is technically still a supragingival plaque [3]. The microbiology of this biofilm has been the subjects of numerous investigastions over the years, however, only recently have the problems associated with sampling of the infected underlying dentine been

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identified and addressed [5-7]. While there is ample evidence to imply a strong association between mutans group streptococci and coronal caries [1-3,5,6], similar data on microbiological agents in root caries is poorly understood.

Some epidemiological studies have shown that *Actinomyces* spp. are predominant bacteria in root caries [1-3,6-9], while other have demonstrated a variety of bacteria and failed to implicate any single bacterial genus as a predominant isolate [3,6,9]. As no data are available on the microbiota of root caries lesions in Polish population, the main aim of study was to evaluate qualitatively the microbiology of root caries in middle- aged and older adults living in Białystok.

Material and methods

Samples of root carious lesions from a total 78 subjects (51 females and 27 males; age range: 35-72 years), provided by dentists practicing in the Białystok, were examined. Among them, 52 (66.7%) adults were classified to middle-aged group 35-44 years (mean 39.6 years; 35 females and 17 males) and 26 (33.3%) to older group 55-72 years (mean 64.3 years; 16 females and 10 males). Samples were taken from selected teeth of subjects as parts of ongoing dental therapy and transferred to a transport medium. All samples were processed within 2 hours of sampling. Transport tubes were incubated in 37°C for 15 minutes and vigorously mixed for 20 to 30 seconds using a vortex mixer. Dilution aliquots of 100 μL were distributed onto two Brucella blood agar plates; 1 plate was used for aerobic incubation for 2 to 5 days at 37°C and the other plate was used for anaerobic incubation using Gas Pak system (Becton-Dickinson) for 2 to 7 days at 37°C. Selective media such as McConkey, Chapman, Cetrimide, Rogosa agar (for *Lactobacillus* spp. and *Bifidobacterium* spp.) and Sabouraud glucose agar for yeasts (*Candida* spp.) were also inoculated.

Charcoaled paper points, as well as the remaining fluid, were transported to a semiliquid medium for anaerobic bacteria and incubated at 37°C for up to 14 days. In cases of no growth on the agar plates, tubes were checked daily for turbidity during 14 days.

Bacteria growing on agar plates were preliminarily identified based on colony morphology, Gram stain and oxygen tolerance. Isolates were further identified into genus and/or species based on selective media and API system (API Staph, API Strep, API NH, API Coryne, API 20E, API 20 A, API 20 NE and API 20C AUX) (bioMérieux) [10,11].

This study was approved by the Bioethics Committee of the Medical University of Białystok. Informed consent was obtained from all participants.

The isolation frequency of microorganisms in the different groups of examined subjects were analysed with the Chi-squared test.

Results

The analysis of results was performed independently in two age-groups of examined adults, i.e. 52 (66.7%) subjects aged 35-44 years (middle age) and 26 (33.3%) – aged 55-72 years (older age) (*Tab. 1*). There were 120 bacterial strains isolated from root carious lesions in middle-aged subjects, 63 (52.5%) strains belonged to 5 genera of aerobic bacteria and 57 (47.5%) – to genera of anaerobic bacteria ($p>0.05$). While in the second group, 85 strains were isolated, 54 (63.5%) – 6 genera of aerobic bacteria and 31 (36.5%) – 4 genera of anaerobic bacteria ($p=0.0004$) (*Tab. 1*).

Among all isolated microorganisms, *Candida* spp. were compromised about 10% in both examined groups (17/137; 12.4% vs 8/93; 8.6%) ($p=0.3627$) (*Tab. 1*).

There were no differences between the isolation rate of a various species or genera in both examined groups, except for *Streptococcus* spp. (12/52; 23.1% vs 19/26; 73.1%) ($p=0.001$), *Streptococcus oralis* (1/52; 1.9% vs 7/26; 26.9%) ($p=0.0006$), *Micrococcus* spp. (0 vs 2/26; 7.7%) ($p=0.04$), *Neisseria* spp. (7/52; 17.3% vs 12/26; 46.2%) ($p=0.007$) and *Veillonella* sp. (8/52; 15.4% vs 9/26; 34.6%) ($p=0.053$), which statistically significantly most frequently occurred in elderly (*Tab. 2*).

Table 1. **Number of bacterial and fungal strains isolated from root carious lesions in adult patients**

Microorganisms	35-44 years (n=52)	55-72 years (n=26)	Total (n=78)
I. Aerobic bacteria	**63**	**54**	**117**
1. Gram-positive	49	39	88
Gram-positive cocci	49	39	88
Streptococcus spp.	12	19	31
Gemella morbillorum	2	-	2
Staphylococcus spp.	35	18	53
Micrococcus spp.	-	2	2
2. Gram-negative	14	15	29
Gram-negative cocci	9	12	21
Neisseria spp.	9	12	21
Gram-negative rods	5	3	8
Haemophilus parainfluenzae	5	2	7
Escherichia coli	-	1	1
II. Anaerobic bacteria	**57**	**31**	**88**
1. Gram-positive	45	22	67
Gram-positive cocci	40	19	59
Peptococcus spp.	6	-	6
Peptostreptococcus spp.	34	19	53
Gram-positive rods	5	3	8
Actinomyces spp.	3	2	5
Bifidobacterium spp.	-	1	1
Lactobacillus spp.	1	-	1
Propionibacterium spp.	1	-	1
2. Gram-negative	12	9	21
Gram-negative cocci	11	9	20
Veillonella spp.	11	9	20
Gram-negative rods	1	-	1
Bacteroides caccae	1	-	1
Bacteria:	120	85	205
Gram-positive	94	61	155
Gram-negative	26	24	50
III. *Candida* spp.	**17**	**8**	**25**
Total	**137**	**93**	**230**

***Table 2*. Isolation frequency (%) of microorganisms from root carious lesions in adult patient according to age**

Microorganisms	35-44 years (n=52)	55-72 years (n=26)	Total (n=78)
Streptococcus spp.	*23.1	*73.1	39.7
S. intermedius	1.9	3.8	2.6
S. mitis	5.8	15.4	9.0
S. oralis	*1.9	*26.9	10.3
S. salivarius	3.8	7.7	5.1
S. sanguis	3.8	3.8	3.8
S.vestibularis	5.8	15.4	9.0
Gemella morbillorum	3.8	0	2.6
Staphylococcus spp.	67.3	69.2	67.9
S. aureus	3.8	3.8	3.8
S. caprae	1.9	0	1.3
S. capitis	3.8	7.7	5.1
S. epidermidis	34.6	42.3	37.2
S. haemolyticus	1.9	0	1.3
S. hominis	3.8	7.7	5.1
S. saccharolyticus	1.9	0	1.3
S. simulans	7.7	3.8	6.4
S. warneri	7.7	3.8	6.4
Micrococcus spp.	0	*7.7	2.6
Neisseria spp.	*17.3	*46.2	26.9
N. flavescens	3.8	7.7	5.1
N. mucosa	1.9	7.7	3.8
N. sicca	3.8	11.5	6.4
N. subflava	7.7	19.2	11.5
Haemophilus parainfluenzae	9.6	7.7	9.0
Escherichia coli	0	3.8	6.4
Peptococcus spp.	11.5	0	7.7
Peptostreptococcus spp.	65.4	73.1	67.9
Bifidobacterium spp.	0	3.8	1.3
Lactobacillus spp.	1.9	0	1.3
Propionibacterium spp.	1.9	0	1.3
Actinomyces spp.	5.8	7.7	6.4
A. naeslundii	3.8	7.7	5.1
Actinomyces sp.	1.9	0	1.3
Veillonella spp.	21.2	34.6	25.6
V. parvula	5.8	0	3.8
Veillonella sp.	*15.4	*34.6	21.8
Bacteroides caccae	1.9	0	1.3
Candida spp.	32.7	30.8	32.1
C. albicans	26.9	30.8	28.2
C. lusitaniae	1.9	0	1.3
C. pelliculosa	1.9	0	1.3
C. pulcherrima	1.9	0	1.3

* $p<0.05$

The yeast of *Candida* genus of 4 species (*C. albicans*, *C. lusitaniae*, *C. pelliculosa*, and *C. pulcherrima*) were isolated from middle-aged subjects (17/52; 32.7%) a comparable rate to older adults (8/26; 30.8%; only *C. albicans*) (p=0.8638) (*Tab. 2*).

Discussion

It is thought that the root carious lesion occurs as a function of accumulation and subsequent stagnation of a plaque biofilm at the gingival margin [3]. The nature of the microbiota is poorly understood, but there seems to be no single species responsible for disease and progression [3,6,9]. What may be important is the presence of particular strains of a defined but heterogenous group of bacteria that are particularly suited to that environment [3]. Indeed, Sansone et al. [9] have shown that the acidogenic and aciduric flora associated with a carious lesion is far more diverse (approximately 25 taxa) than the corresponding acidogenic and aciduric flora associated with sound root surfaces (approximately eight taxa).

Beighton and Lynch [5] showed that the bacterial composition of the carious dentine biofilm associated with "soft" lesions consists of significantly more lactobacilli and Gram-positive pleomorphic rods and conversely, significantly fewer streptococci compared with the overlying plaque biofilm. Additionally, there is an increased number and/or proportion of *S. mutans* in "soft" lesions compared with "hard" lesions or sound surfaces. The lesion has been shown to have a definite progression, since changes in its clinical appearance are observed over time [3,5].

Actinomyces spp. have historically been associated with root surface caries, although the nomenclature of the species and genospecies in the literature confuses the matter greatly; see Johnson et al. [7]. However, in recent studies by Brailsford et al. [8,12] *Actinomyces naeslundii* was shown not to be associated with active carious lesions and that *A. israelii* and *A. gerencseriae* predominated. Shen et al. [13] also isolated three different *Actinomyces species*, including *A. israelii*, *A. meyeri* and *A. odontolyticus*, amongst which *A. israelii* was most predominant. The predominant microorganisms isolated by Shen et al. [13] from 30 root caries lesions were *Lactobacillus* spp. (90%), *Streptococcus* spp. (100%) and *Actinomyces* spp. (63%). This result of the present Chinese subject was in agreement with studies, most of which conducted on Caucasians subjects, at other parts of the world [14,15]. More recent studies on root carious lesions also indicated that predominant cultivable flora were streptococci, lactobacilli, *Actinomyces* spp. and staphylococci [16,17].

In the present study, we isolated only 5 (6.4%) strains of *Actinomyces* spp. from 78 carious lesions of adult subjects. Among them, 4 strains belonged to one species of *Actinomyces naeslundii*. This species was absence in 30 such lesions in elderly institutionalized, ethnic Chinese [13]. From our subject examined also not frequently was isolated *Lactobacillus* spp. (only one strain). The predominant microorganisms isolated from root carious lesions were *Streptococcus* spp. and *Peptostreptococcus* spp. (73.1% each) in older adults, while *Staphylococcus* spp. and *Peptostreptococcus* spp., as well as in middle-aged (67.3% and 65.4%, respectively) and overall subjects studied (67.9% each genus).

Shen et al. [13] isolated from root caries of elderly population also *Staphylococcus* spp. and *Peptostreptococcus* spp., and other taxa such as *Veillonella* spp. and *Candida* spp. The isolation frequency of *Veillonella* spp. in our study was 25.6%, and was comparable between older (34.6%) and middle-aged (21.2%) subjects (p=0.1993). The isolation frequencies of *Veillonella* spp. in root caries represented by different researches vary. In two reports Ellen et al. [14] and van Houte et al. [16] recorded 96% and 42-100% isolation frequency of *Veillonella* spp., respectively, from root caries. On the contrary, in our study

(25.6%) and in study performed by Shen et al. [13] (26.7%), was relatively low. From the host point of view, *Veillonella* spp. are considered beneficial organisms in the carious process as they metabolize short-chain carboxylic acids produced by neighbouring cariogenic flora [1,2].

The role of staphylococci in caries initiation and development is not well known. The recent study by Schupbach et al. [6] reported the proportions of staphylococci in initial and advanced lesions as 4.4 and 15.5%, respectively. In our study, 25.9% of the total bacterial isolates were identified as *Staphylococcus* spp.

Peptostreptococcus spp. are member of the normal oral microbiota, but some *Peptostreptococcus* species are thought to be associated with anaerobic infections including gingivitis and periodontitis [18].

In previous cohort studies of middle-aged healthy adults living in Białystok we have demonstrated, in general, a 41% oral carriage rate for yeasts [19]. The high isolation frequency (32.1%) of *Candida* spp. from root caries lesions of adult subjects in our study may suggeste that carious foci are reservoirs of yeasts.

There were no significant differences in root caries microbiology between denture wearers (22: 11 middle-aged and 11- older adults) and non-denture wearing subjects (56: 41 middle-aged and 15-old-age) (data not shown). These differences were observed by Shen et al. [13]. Notably, significantly more Gram-positive cocci, Gram-negative rods, *Staphylococcus* spp. *Actinomyces* spp. and *Candida* spp. ($p<0.05$) could be isolated from the non-denture wearing group [13].

In conclusion, the present study provides baseline information on the microbiologic features of root caries in the middle--aged and elderly subjects in the north-eastern region of Poland.

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