Identification of infectious microbiota from oral cavity environment of various population group patients as a preventive approach to human health risk factors

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Abstract

Introduction and objective. This study presents the results of comparative investigations aimed to determine microbiota that can occur in the oral environment in different human populations. The objective of the research was to identify pathogenic oral microbiota, the potential cause of health complications in patients of different population groups.
Materials and method. The study included 95 patients requiring dental or surgical treatment; their oral cavity environment microbiota as risk factors of local and general infections were assessed.
Results. In clinical assessment, differences occurred in oral cavity conditions between patients with malformations of the masticatory system, kidney allograft recipients and individuals without indications for surgical procedures. The presence of various pathogenic and opportunistic bacterial strains in oral cavities were revealed by direct microscopic and *in vitro* culture techniques.
Conclusions. Colonization of oral cavities of patients requiring surgical treatment by the potentially pathogenic bacteria constitutes the threat of their spread, and development of general infections. Assessment of oral cavity infectious microbiota should be performed as a preventive measure against peri-surgical complications.

Key words

infectious microbiota, oral cavity, risk of peri-surgery complications, preventive approach

INTRODUCTION

The oral cavity is a morpho-physiologically heterogeneous, dynamic environment with biotic and abiotic factors influencing with different intensity various specialized surfaces of its particular compounds (tongue, teeth, gums, etc.). Among these factors, temperature, pH of saliva (related to food substances), oral care agents and specific diet may impact oral cavity ecology. This environment creates an open system with dynamic ecological conditions promoting its colonization with many microbiota and influences their species composition. Resident species of the Gram positive *Streptococcus viridans* group are believed to be typical oral bacteria – inhabitants of healthy oral cavities, not related to any specific clinical pathological symptoms [1, 2, 3, 4, 5, 6]. The oral cavity microbiome consists of a multi-species community with its complex relations to the human host. Single cells and their micro-colonies creating biofilm remain in a labile homeostasis; simultaneously, human immunological system activity inhibits microbiota multiplication [7, 8, 9]. Numerous

factors, both environmental and hereditary, may influence the development of an oral pathology. An occupational specificity of generally healthy persons causing difficulties with the maintenance of good oral health, unfavorable economic, social, sanitary circumstances, and, also systemic disorders and congenital malformations of the masticatory system, are factors influencing poor oral hygiene and contributing in the appearance of dental difficulties [6, 10, 11, 12, 13]. To-date, dental caries and periodontal diseases are major health problem in Poland [2, 14, 15, 16].

The majority of studies on oral microbiota regard bacterial species related with social diseases: caries (e.g. streptococci: *S. mitis*, *S. oralis*, *S. salivarius*) and inflammatory periodontal diseases (from genera *Actinobacillus, Porphyromonas, Campylobacter*); recently, *Scardovia wiggsiae* has also been considered as an important etiological agent of early childhood caries [2, 15, 16, 17]. Changes in the masticatory system and disturbances of labile oral microbiome homeostasis that impact oral ecology and microbial composition may promote a penetration and colonization of the mouth with other exogenous species, including the potentially pathogenic.

It is emphasized that bacterial community components immersed in a polysaccharide matrix adhering to human tissues as a biofilm are less susceptible to human immunological system activity, to antibiotics, and antiseptics,

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Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 4

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in comparison to free bacteria out of biofilm; thus, biofilm-related infections may cause treatment complications [18].

To-date, the coexistence of various microbiota species in the oral cavities of individuals with different local and systemic disorders, has not received enough attention and remains a rare subject of studies. Previous interdisciplinary studies by the authors of the presented study revealed that various metabolic disabilities have a different influence on species diversity of oral cavity microorganisms [5, 6, 12, 15, 19, 20, 21, 22]. This study is the subsequent comparative investigation aimed at determining the microbiota that can occur in the oral environments in different human populations.

OBJECTIVE

The aim of the presented study was to compare identified pathogenic oral microbiota, the potential cause of health complications in patients of different population groups. This interdisciplinary work pertains to individuals with and without systemic diseases requiring dental or surgical treatment, and to their oral cavity environment microbiota as risk factors for local and general infections.

MATERIALS AND METHOD

Clinical assessment of patients. The retrospective study included 95 patients treated conservatively or surgically between 2006–2015 in Clinics of the Medical University of Warsaw. The patients, men and women who ranged from 20–55 years of age, were classified into 3 groups:

Group I involved thirty patients with congenital malformations of masticatory system (after surgically closed cleft lip and palate);

Group II involved 30 kidney allograft recipients (under immunosuppression after renal transplantation);

Group III – the control group, included 35 dental patients preservatively treated, without indications for surgical procedures.

Sixty patients with systemic diseases and 35 without serious disorders, evaluated for their oral health status were analyzed.

The patients, before further treatment, underwent clinical assessment for health status of their periodontium, gingiva, presence of inflammatory processes, and treatment of tooth decay.

Isolation and identification of oral cavity microbiota. Swabs from 10 sites of the periodontium, dental plaques, and from dental pockets were collected from each patient for qualitative assessment of microbiota. Wet and Giemsa stained smears were prepared for light microscopy for the preliminary identification of Gram- positive and Gram -negative bacteria strains. Conventional *in vitro* culture techniques were also applied for bacteria specific identification [2, 4]. The swab material was grown aerobically on bacteriological agar and on agar with 5% defibrinated sheep blood. Chapman's plate growth medium for recovery and isolation of *Staphylococcus* strains and McConkey's medium for identification of Enterobacteriaceae were applied. Prevalence of bacterial strains detected in the oral cavities of each group of patients was determined and statistically assessed (Statistica, F-Fisher, HSD-Tukey test p<= 0.05).

RESULTS

Oral cavity clinical characteristics. There were differences in oral cavity conditions between all patients' groups.

In the clinical assessment of 30 patients with malformations of the masticatory system (Group I), occlusion anomalies, cross-bites, and prognathism, retrognathism, dental discrepancies occurred. In scars and fissures left after surgical closing of cleft lip and palate, accumulated saliva and food debris were very frequently visible.

In 30 kidney allograft recipients (Group II), occlusion anomalies, loose teeth, gingival bleeding, and periodontitis occurred; mucosal inflammation and drug-induced gingival hyperplasia due to immunosuppressive medication were also observed.

All patients of these two groups were affected by caries.

Clinical examinations of 35 patients without systemic disorders treated conservatively (Group III), without indications for surgical procedures, showed a generally better status of their oral cavities; however, loose teeth, gingival bleeding, dental caries, and gingivitis were noted in some cases.

Microbiota isolated from oral environments of particular population groups. Examinations of microscopic and *in vitro* cultured samples of the material obtained from oral cavity swabs obtained from particular patient groups showed the presence of various pathogenic and opportunistic bacterial strains in the superficial layer of periodontium and in dental pockets.

Typical oral Gram- positive bacteria of the *Streptococcus viridans* group were identified in all patients. Apart from resident species, in the oral cavities of the examined individuals other bacterial strains occurred that belonged to 6 Gram- positive and 8 Gram- negative bacteria species.

Comparative qualitative analysis revealed significant differences in the prevalence of bacterial strains detected in the oral cavities of particular patient groups. The faecal bacteria *E. faecalis* and *E. faecium* were the most frequently isolated from the oral cavities of all patient groups.

S. aureus was more frequently isolated from the oral cavities of patients of Group I and Group II, than of Group III.

Opportunistic and pathogenic bacteria strains isolated from the oral cavities of particular patient groups are presented in Table 1.

Prevalences of selected pathogenic bacteria species in patients with malformations of masticatory system, kidney recipients and persons without systemic disorders, are presented in Table 2. As seen in the Table, the prevalences of *E. faecalis/ E. faecium*, *S. aureus* and *E. coli* in the oral cavities of patients of Group I were significantly greater (p<0.05), compared to Group III, and in the case of *E. coli,* also compared to Group II. The prevalence of *E. faecalis/ E. faecium* in the patients of Group II was significantly greater compared to Group III (Tab. 2).

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Table 1. Opportunistic and pathogenic bacteria strains isolated from oral cavities of particular patients groups

Gram-positive bacteria strains	Isolated from patient groups	Gram-negative bacteria strains	Isolated from patient groups
Enterococci		Enterobacteriaceae	
Enterococcus faecalis	I, II, III	*Enterobacter cloacae*	I, II, III
Enterococcus faecium	I, II, III	*Escherichia coli*	I, II, III
		Pantoea agglomerans	I
		Klebsiella pneumoniae	I, II
		Klebsiella oxytoca	I, II
		Proteus mirabilis	I
Micrococcus luteus	I, II	non-Enterobacteriaceae	
Bacillus sp.	I, II, III	*Pseudomonas aeruginosa*	I
		Acinetobacter baumannii	I
Staphylococci			
Staphylococcus aureus	I, II, III		
Staphylococcus epidermidis	I, II, III		

Table 2. Comparison of prevalences of selected pathogenic microbiota from oral cavity of patients from particular groups.

Bacteria strains	Patients with the oral microbiota		
	Number / % Group I	Number / % Group II	Number / % Group III
Gram- positive			
E. faecalis	15/ 50%***	12/ 40%**	3/ 8.8%
E. faecium			
Staphylococcus aureus	9/30%*	4/ 13.3%	2/ 5.7%
Micrococcus luteus	3/ 10%	2/ 6.6%	0 -
Gram- negative			
Enterobacteriaceae			
Escherichia coli	15/ 50%*** ##	3/ 10%	2/ 5.7%

*-*** significantly greater compared to Group III; *p<0.05; **p<0.01
***p<0.001; ## significantly greater compared to Group II: p<0.01

DISCUSSION

Components of oral cavity microbiota and the coexistence of particular strains in the oral cavity environments are more frequently investigated. Associations between the occurrence of the infectious bacteria, protozoans and fungi, and oral hygiene, various local/ general metabolic deteriorations and chronic diseases, are the important objective of investigations, also undertaken by the authors of the current study [5, 6, 15, 20 – 23].

The results of this analysis of retrospectively evaluated mouth microbiota of different population groups, indicated that serious systemic diseases requiring surgical treatment, and permanent medication, including chronic immunosuppression, can impact oral cavity environments. This was expressed, among others, by the colonization of disordered patient oral cavities with exogenous infectious microbiota, and the dissemination of endogenous, potentially pathogenic bacteria strains from deeper parts of the intestinal tract into the mouth.

Among Gram-positive bacteria strains, *Enterococcus faecalis* and *E. faecium*, common in the human large intestine and sporadically found in the healthy mouth, were frequently found in the oral cavities of the analyzed population groups, and in earlier investigated patients with masticatory system disorders [6, 15, 22]. The bacteria may act as opportunistic agents of serious, life-threatening infections especially dangerous for immune-compromised and elderly persons; some strains of the bacteria have been isolated with the increasing frequency in hospital-acquired diseases, e.g. stomatopathy, pneumonia, and nosocomial urinary tract infections [2, 4]. Saprophytic bacteria of *M. luteus* that contaminate skin and mucosa of persons with weakened immune system, may be opportunistic strains and causative agents of serious pneumonia, septic arthritis, endocarditis, and even meningitis; they are also implicated as etiological agents in cases of infections associated with surgical procedures [4]. Gram- negative bacteria strains of *E. coli* may cause respiratory and urinary tract infections, severe diarrhea in children and adults, particularly in individuals with weakened immune systems. Several strains of *E. coli* rods: enteropathogenic, enterotoxic and enterohemorrhagic, may be the causative agents of nosocomial infections and sepsis. *S. aureus* strains, including MSRA-methycillin-resistant are known to be responsible for difficult-to-treat infections in humans. *Staphylococcus epidermidis* may be a causative agent of gingivitis and endodontic infections.

Knowledge about the composition and local and systemic effects of the presence of microbiota that can colonize the oral cavity is essential for decreasing the health risks for patients. The technological development, advances in sequencing tools, new data on polymicrobial infections, and on uncultivable organisms, influence changes our understanding of the oral microbiota and its role in oral-dental health and disease. According to Lunsford et al. [24]: 'Following many decades of culture-dependent studies involving the oral microbiota, metagenomic projects like the HMP (the US National Institutes of Health launched the Human Microbiome Project) have confirmed that the oral cavity is one of the most taxonomically-diverse body sites, second only to the gut, in overall microbial complexity'. The human oral cavity is inhabited by hundreds of bacterial species, some of which have a key role in dental caries and periodontitis. However, Belda-Ferre et al. [25] underlined in their culture -dependent and molecular-based studies expanding the list of cariogenic bacteria, that synergistic effects probably take place and that caries is a poly-microbial disease. There are evidences for a great degree of variation in the diversity of mouth community structures associated with oral cavity health status [26, 27, 28].

The results of the studies by the authors of the presented study exhibit the usefulness of *in vitro* culture technique-dependent studies for the identification and elucidation of a diversity of oral cavity bacteria occurring in kidney allograft recipients, and patients with malformations of the masticatory system.

The impact of the main disease in these patients was expressed by a pronounced shift in the microbiota species composition, and also in the number of particular strains in comparison to these that have been detected in generally healthy persons under dental treatment.

The study on oral microbiota was also conducted to determine associations between oral hygiene and periodontal

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health, and chronic obstructive pulmonary disease (COPD) exacerbations [29, 30]. The study demonstrated a significantly higher number of potential pathogenic microorganisms in the denture plaque of patients with COPD, compared to healthy individuals [29]. It was also emphasized that improvement of periodontal health and oral hygiene may be a potentially preventive strategy against COPD exacerbations [30].

CONCLUSIONS

The results of this comparative analysis revealed significant differences in the composition of the oral environment multi-species microbiota community between patients groups requiring surgical treatment, and the generally healthy persons from the control group. Numerous pathogenic and opportunistic bacterial strains detected from the dental pockets and from superficial layers of periodontium of both assessed patient groups, may act as etiological agents of human infectious diseases. Colonization of the oral cavity by potentially pathogenic bacteria constitutes the threat of their transmission to various human tissues and organs. Thus, there is an increased risk of the development of general infections, which would be particularly serious for persons with impaired immunity; such a health risk should be taken into consideration. Assessment of the oral cavity environment infectious microbiota should be performed not only as a preventive measure against the peri-surgical complications, but also in support oor the management of surgical treatment efficacy.

Acknowledgements

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