

## MICROBIOLOGICAL RISK FACTORS IN DENTISTRY. CURRENT STATUS OF KNOWLEDGE

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**Abstract:** Dentists belong to a professional group potentially exposed to harmful biological factors which most often are infectious microorganisms, less frequently - allergenic or toxic microorganisms. The fundamental routes of spreading harmful microorganisms in a dental surgery are: blood-borne, saliva-droplet, direct contact with a patient and with infected equipment, and water-droplet infections. In this paper, the current status of knowledge on microbiological hazards in a dentist's work is presented. Groups of microorganisms, such as prions, viruses, bacteria, fungi and protozoa, to which a dentist is, or may be exposed, are discussed. Epidemiological assessment of microbiological hazards in a dentist's work was performed and the basic principles of prevention formulated. Special attention was given to microflora in dental unit waterlines, and the biofilm persisting in them, as a source of occupational hazards specific for a dentist's workplace.

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### SOURCES OF MICROBIOLOGICAL RISK FACTORS

In their work, dentists are exposed to contact with microorganisms harmful to health. Most often they are infectious microorganisms; allergenic and toxic ones are less common. The source of harmful factors is, in most cases, the patient, but it may also be the water used in a dental unit. There are 4 basic routes of spreading harmful microorganisms in a dental surgery:

- blood-borne route - through the blood of an infected patient;
- saliva-droplet route - through a droplet aerosol, emitted by an infected patient and containing particles of saliva, secretions from the gum, periodontium and teeth;
- direct contact with a patient and contaminated equipment;
- water-droplet route - through a water droplet aerosol emitted from a handpieces of a dental unit which may

contain microorganisms present in a unit reservoir, or developing in biofilm inside a unit tubing.

The greatest risk for the dentist are viruses spread through blood (hepatitis B and C viruses, HIV virus) which cause serious health and life-threatening diseases [7, 34]. The risk from these viruses has been the subject-matter of numerous publications [12, 13, 18, 35, 38] and to a large extent, it is identified with biological hazards in dentistry [1, 33].

Risk factors spread via the saliva-borne route and through direct contact include a vast range of microorganisms, from prions through viruses and bacteria to fungi. *Herpes simplex* virus is among the important risk factors transmitted in this way [23].

The best known disease factors are spread by spraying water from dental units contaminated with microorganisms. Among the infectious microorganisms, bacteria from *Legionella* species developing inside dental unit

waterlines constitute the highest risk [3, 31, 61]. Gram-negative rods producing allergens and endotoxin are important risk factors among the potentially allergenic and toxic microorganisms. To date, extensive research of water used in dental units, and of water aerosol, to allow a comprehensive evaluation of hazards in dentistry in this respect, has not been conducted.

## MICROBIOLOGICAL RISK FACTORS REVIEW

### Prions

Prions are particles of mutated protein which may cause a spongiform encephalopathy in humans (Creutzfeldt-Jakob disease) and in animals (BSE, scrapie). The disease is usually transmitted through infected feed, but other routes of infection which have not been fully understood, are also considered. One of the hypotheses assumes the possibility of infection during a dental procedure. The hypothesis is based on the results of experiments in which infectious prions were found in salivary glands, tooth pulp, trigeminal nerve and lymphatic glands of laboratory animals [46, 50]. The risk of infection is also increased by the emergence in recent decade in the UK, of a rapidly developing variant of Creutzfeldt-Jakob disease (vCJD), occurring - contrary to the typical form of the disease - and mainly in young people [17]. Due to these circumstances, dentists treating patients with a diagnosis of Creutzfeldt-Jakob disease are recommended to take special precautions to protect themselves and other patients [45, 50, 62]. Considering an exceptionally high resistance of prions to high temperature and sterilization, it is even recommended to irretrievably discard dental instruments used in such cases [46]. However, no case of transmission of a prion-caused infection during a dental procedure has yet been recorded.

### Viruses

#### Blood-borne viruses

**Hepatitis B virus (HBV).** This virus constitutes a particular hazard for dentists because the disease is easily transmitted and has grave consequences. HBV viruses contain DNA. They are highly infectious, easily transmitted through blood and saliva, and highly resistant to environmental agents, disinfection and sterilization. The dentist may become infected with HBV from a patient both through needlesticks or other accidental percutaneous injuries with sharp instruments, and through an aerosol of blood, saliva or gingival secretion. Viral antigen (HbsAg) was found in 76% of saliva samples taken from the virus carriers, and the site of the highest concentration of the virus in the oral cavity is the gingival sulcus [12]. It is estimated that in the case of an injury with a HBV contaminated needle, the probability of infection is 6-30% [7, 35]. The virus causes asymptomatic infections, acute or chronic, which may lead to cirrhosis

or primary liver cancer. HBV is assessed to be the second, after tobacco, most frequent cause of cancer [13].

It is estimated that infections with type B viral liver inflammation occur in dentists at least 3 times more often than in the general population, and in dental surgeons - at least 6 times more frequently [12, 13]. According to other sources, dental care professionals are exposed to 10 times higher risk of infection with chronic hepatitis B than an average individual [1]. Until recently, the disease has decidedly been the most common occupational infectious disease in dentists, and in other health care professionals. General vaccination of health care personnel and introduction of disposable syringes and needles considerably reduced the number of occupational infections with hepatitis B in this professional group, both in Poland and worldwide [12, 43, 44]. However, HBV still remains a very serious hazard for health care workers, including dentists [12, 18, 44].

**Hepatitis C virus (HCV).** HCV is a blood-borne virus containing RNA, epidemiologically similar to HBV. Hepatitis C is a serious health problem because in ca. 90% of cases, infection is asymptomatic; it may develop into a chronic liver inflammation and eventually progress to cirrhosis and chronic liver cancer [19, 35]. The common phenomenon of virus carriage (asymptomatic infection), occurring both in hepatitis C and hepatitis B, is epidemiologically unfavourable and significantly increases the risk for health care personnel, including dentists [34, 35].

HCV is less infectious than HBV, because in the case of an injury with a contaminated needle, the infection develops only in 1.8-6.0% of cases [7, 35]. Due to the fact that a vaccine against HCV has not been produced as yet, the incidence of occupational hepatitis C in health care professional shows a tendency to increase, and in Poland it has already exceeded the number of hepatitis B cases [43, 44]. In Poland, management of both types of liver inflammation is obstructed by the poor condition of sterilization and disinfection utilities in health care centres [19].

Hepatitis C is a serious hazard also for dentists, although infection risk is lower than in the case of hepatitis B [1]. On the other hand, patients with hepatitis C are more prone to extensive dental diseases, which increases the risk for dental professionals [38]. After procedures on patients with hepatitis C, HCV genetic material was found on dental instruments and dental office equipment [38]. The hazard for a dentist becomes greater also due to the presence of HCV in the saliva of an infected patient. Matičič *et al.* [30] found, using the PCR test, HCV RNA in 35% of saliva samples and 59% of gingival secretion samples taken from patients with hepatitis C. According to the authors, saliva may become infected with the virus not only through blood but also through gingival fluid.

**Other hepatitis viruses.** Blood-borne, RNA-containing viruses causing hepatitis D (HDV) and hepatitis G (HGV) may also constitute occupational hazards for dentists [18,

35]. HDV is a virus associated with HBV (HDV = Delta + HBV) [16, 52], while HGV is a virus related to HCV, but occurring separately. It is believed that HGV is less infectious and virulent than HBV and HCV, but not all characteristics of this virus are sufficiently known [18, 27]. Preliminary results of epidemiological studies show high HGV prevalence in dental professionals [27].

**Virus causing AIDS disease (HIV).** HIV virus, causing the acquired immunodeficiency syndrome (AIDS) is an RNA-containing retrovirus, transmitted in a blood-borne mode and through sexual contact. It attacks human CD-4 lymphocytes, causing a drastic immunity drop and death, most frequently as a result of infection with opportunistic microorganisms, normally indifferent to human health. Some of these infections start in the oral cavity, which increases the risk for a dentist [29].

HIV concentration in serum is 10-1,000/ml and is on average 10,000 times lower than that of HBV [35]. The virus is very sensitive to unfavourable physical and chemical factors in the outside environment. In consequence, in case of an injury with a HIV-contaminated needle, the probability of infection is only 0.3% [7, 35].

A very low infectivity of the virus is epidemiologically favourable and explains the small number of registered occupational cases of AIDS in health care workers, including dentists, despite the high exposure connected with an increase of the disease incidence in many countries [1, 29]. Nevertheless, dentists should adopt extensive safety measures when treating suspected AIDS patients or people from at-risk groups.

#### **Viruses transmitted in a saliva-droplet mode and through direct contact**

**Herpes simplex viruses (HSV-1, HSV-2).** *Herpes simplex* viruses of type 1 (HSV-1) and 2 (HSV-2), causing a recurrent labial and genital herpes, herpetic whitlow and keratitis represent a particular hazard for dental workers because of their common occurrence and high infectivity. It is assumed that the incidence of HSV-1 infection, which is asymptomatic in the vast majority of cases, amounts to ca. 90% in people of 60 years of age [23]. In the asymptomatic phase, the virus concentrates mainly in the trigeminal nerve, although it was found during this phase in saliva in 4.7% of the population [49]. The disease can be reactivated by various agents, including surgical procedures in the oral cavity. At this time, oral ulceration may occur, particularly in the gums, hard palate, and labial and cheek mucous membranes. In this phase, lasting 1-4 days, HSV virus was isolated from 89% of samples taken from oral ulcers and from 25% of saliva samples [49]. According to Lewis [23], dentists in contact with HSV-1 patients are most frequently infected with herpetic whitlow, whose incidence in this group is significantly higher than in the general population, and with keratitis.

**Virus causing SARS disease (SCoV).** The virus causing severe acute respiratory syndrome (SARS) is a particularly virulent, newly-discovered coronavirus (SCoV) containing RNA, spreading in a droplet mode [17, 24]. Presumably, the SCoV reservoir are animals (civets, genets, badgers); however, this hypothesis still requires confirmation. At the end of 2002, in south-eastern Asia, the virus caused a violent epidemic of a disease defined as SARS, with the characteristics of an atypical pneumonia, which lasted over half a year and, during this period, led to over 8,400 disease cases and over 900 deaths in 32 countries [24]. It should be particularly stressed that health care workers were over 25% of the patients, and in some cities, e. g. Hong Kong and Toronto, they constituted over a half of all the infected with SARS [17]. This shows that health care workers are placed under a particular threat from new viruses that emerge in various parts of the world and may cause violent, potentially recurrent epidemics. The emergence of such a virus requires adopting special preventive measures including isolation of patients, air sterilization, rubber dam and other precautions [25].

**Other viruses spreading in a saliva-droplet mode and through direct contact.** The list of viruses which spread in a saliva-borne mode or through direct contact and during the procedure may create a risk of infection for a dentist, is very long and includes over 170 species of viruses causing respiratory system diseases, not to mention species responsible for other diseases [34]. The most important groups and species of viruses constituting an occupational hazard for a dentist are the following: adenoviruses, coronaviruses, cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesviruses type 6-8 (HHV-6, HHV-7, HHV-8), varicella-zoster virus (VZV), influenza virus (type A, B, C), measles virus, parotitis virus, parainfluenza viruses (type 1-4), RS virus (RSV), human parvovirus (B19), coxsackie virus (A21), echo viruses 6 and 20, polio virus, other enteroviruses, rhinoviruses, reoviruses, rubella virus [4, 16, 34, 52, 62].

A high exposure of dental workers to contact with pathogenic viruses is confirmed by the research results: antibodies against 4 viral antigens: influenza A and B viruses, RS virus and adenoviruses were found in 68% of studied dentists. The difference between the frequency of antigens occurrence in the group of dentists and in the non-exposed control group proved highly significant ( $p < 0.01$ ) in the case of influenza and RS viruses [4].

Potential risk of exposure of dentists to viruses occurring in the oral cavity and causing childhood illnesses should be stressed: in adults with specific immunodeficiency, these diseases may cause complications dangerous to health. These viruses include: varicella-zoster virus (VZV), measles virus, parotitis virus, RS virus, polio (Heine-Medina disease) virus and rubella virus (*Rubivirus hominis*) which can cause damage to the foetus, thus creating a particular risk for a female dentist in the period of pregnancy [4, 16].

Special risk factors for dentists are herpesviruses (Herpesviridae family) [4]. Apart from the above-discusses viruses of *Herpes simplex* (HSV-1, HSV-2) and varicella-zoster virus (VZV), mentioned among the viruses causing childhood illnesses, this group includes: cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human herpesvirus type 6 (HHV-6) [4]. The viruses: CMV, EBV, HHV-6, HHV-7 and HHV-8 latently infect peripheral blood leucocytes, which - considering their high prevalence in the population (in the case of CMV, HHV-6 and HHV-7: 40-100%) - significantly increases the risk for dentists [62]. In dentists and students in the later years of dentistry, anti-EBV antibodies occur more frequently than in dentistry students who do not participate in the treatment of patients [62].

### Bacteria

#### Bacteria spread in a saliva-droplet mode and through a direct contact

***Mycobacterium tuberculosis.*** *Mycobacterium tuberculosis* represents a particular risk to dentists [1, 20, 38, 62]. Mycobacteria developing in the pharynx [34], during a procedure are emitted to the air, most often when coughing, together with dried saliva or sputum droplets, called "droplet nuclei" [20]. This creates a risk of occupational tuberculosis for dentists, which has been described in literature [62].

#### Other bacteria transmitted in a saliva-droplet mode.

Bacteria form dental plaque and bacterial flora of the oral cavity is very abundant. According to Miller and Cottone, one saliva droplet may contain 50,000 bacteria belonging to 25 genera [34]. It is assumed that the oral cavity is inhabited by 300–400 species or groups of bacteria, some of which are potential pathogens [59]. These bacteria can be divided into: aerobic and facultative anaerobic species, and anaerobic species. The aerobic and facultative anaerobic bacteria occurring in the oral cavity include: Gram-negative rods (*Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Eikenella corrodens*, *Bordetella pertussis*, *Haemophilus influenzae*, *Actinobacillus actinomycetemcomitans*, *Campylobacter rectus*); Gram-negative diplococci (*Moraxella catarrhalis*, *Neisseria meningitidis*, *Neisseria flavescens*, *Neisseria gonorrhoeae*); Gram-positive rods and coryneform bacteria (*Lactobacillus acidophilus*, *Corynebacterium diphtheriae*); staphylococci (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus* spp.); streptococci (*Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus milleri*, *Streptococcus sanguis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus* spp., *Enterococcus faecalis*, *Enterococcus* spp.); spirochetes (*Treponema pallidum*), mycoplasmas (*Mycoplasma pneumoniae*). The anaerobic bacteria inhabiting the oral cavity include: Gram-negative rods (*Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella melaninogenica*, *Prevotella oralis*, *Prevotella*

spp., *Fusobacterium nucleatum*, *Fusobacterium* spp., *Bacteroides* spp., *Veillonella* spp.); Gram-positive rods (*Arachnia* spp., *Bifidobacterium* spp., *Eubacterium* spp., *Propionibacterium* spp.); streptococci (*Peptostreptococcus micros*, *Peptostreptococcus* spp.); spore-forming rods (*Clostridium* spp.); actinomycetes (*Actinomyces viscosus*, *Actinomyces israelii*, *Actinomyces* spp.) [9, 10, 33, 34, 59].

Bacteria occurring in the oral cavity are the main cause of teeth and periodontal diseases. Dental caries is usually initiated by acid-producing bacteria of the genera: *Streptococcus mutans*, *Lactobacillus acidophilus* and *Actinomyces viscosus* [9]. Periodontitis is mainly caused by anaerobic bacteria, including *Porphyromonas gingivalis*, *Prevotella intermedia* and *Peptostreptococcus micros*, and also facultative anaerobic bacteria from the species *Actinobacillus actinomycetemcomitans* and *Campylobacter rectus* [59].

From the point of view of the dentist's occupational hazards, common carriage of potentially pathogenic bacteria is an epidemiologically unfavourable phenomenon. It is estimated that the frequency of *Haemophilus influenzae* carriage in the nasopharynx amounts to 33-66% in the general population, of *Neisseria meningitidis* - 3-30%, *Streptococcus pyogenes* - ca. 10%, *Streptococcus pneumoniae* - 20-33%, *Staphylococcus aureus* - ca. 30%. The carriage of *Mycoplasma pneumoniae* is also widespread [34]. Pathogenic bacteria easily penetrate from the nasopharynx to the oral cavity, and next with saliva droplets to the breathing zone air, creating a direct threat to a dentist.

A potential threat to dentists are also some of the Gram-negative bacteria, commonly inhabiting the oral cavity and generally believed to be harmless saprophytes: *Eikenella corrodens*, *Moraxella catarrhalis*, *Neisseria flavescens*. Available case descriptions show that *Eikenella corrodens* can be a cause of submandibular abscess [21] and brain abscess [2], while *Moraxella catarrhalis* and *Neisseria flavescens* can be a cause of bacteraemia [37, 60].

#### Bacteria spread in a water-droplet mode

**Gram negative *Legionella* rods.** *Legionella* rods persisting in water (*L. pneumophila*, *L. bozemanii*, *L. dumoffii*, *L. longbeache*) find a favourable environment for proliferation in biofilms occurring in waterlines of dental units [55]. These bacteria cause legionellosis which may have varying clinical forms. The most dangerous is pneumonia (legionnaires' disease) caused by *Legionella pneumophila pneumoniae* (*Legionella pneumophila* serotype 1), but an influenza-like form of the disease, described as Pontiac fever, is much more common [41].

Inside the waterlines of dental units, *Legionella* rods find optimal ecological conditions for development and biofilm formation, which is due to stagnation of water, its high temperature (23°C) and low concentration of chlorides [55]. These bacteria often develop inside the cells of amoebae which contribute to their transmission [3, 55].

Atlas *et al.* [3] isolated bacteria from *Legionella* genus from as many as 78% of the water samples taken from dental units, including 8% of samples with *Legionella pneumophila pneumoniae*. The concentration of *Legionella* spp. exceeded 1,000 cfu/ml in 36% of samples and 10,000 cfu/ml in 19% of samples, while the concentration of *Legionella pneumophila* never exceeded 1,000 cfu/ml. Williams *et al.* [61] found *Legionella* spp. bacteria in 62% of water samples from dental units, and their concentration in 19% of samples exceeded 100 cfu/ml. In the studies of other, especially German authors, the percentage of water samples contaminated with *Legionella* spp. was lower and fell within the range 10–50% [8, 28, 39, 48]. Research conducted in Poland [31], showed the presence of *Legionella* spp. in 24.2% of tested water samples from dental turbines amounting to 1–200 cfu/ml, and 13% of the isolated strains belonged to the most pathogenic *Legionella pneumophila* serotype 1.

A high prevalence of *Legionella* rods in water of dental units create risk for dentists who are exposed to inhaling droplet-water aerosol containing these bacteria [55, 62]. This is confirmed by the results of some seroepidemiological research which determine that the percentages of positive serological reactions to *Legionella* antigen in dental workers were higher than in the general population [48].

**Gram-negative rods.** Water from dental units may also be contaminated with other Gram-negative bacteria from many various genera which, in their vast majority, come from biofilms occurring inside dental unit waterlines. These bacteria usually come from Pseudomonadaceae family (*Pseudomonas* spp., *Burkholderia* spp., *Brevundimonas vesicularis*, *Sphingomonas paucimobilis*, *Stenotrophomonas maltophilia*). Apart from these, the following Gram-negative bacteria were found in dental unit waterlines: *Acinetobacter calcoaceticus*, *Achromobacter* spp., *Aeromonas salmonicida*, *Alcaligenes faecalis*, *Escherichia coli*, *Flavobacterium* spp., *Moraxella* spp., *Pasteurella* spp. [6, 32, 51, 53, 54, 56, 57, 61, 62]. Among these bacteria, only *Pseudomonas aeruginosa* [6] and *Klebsiella* spp. [62] were infectious, while the remaining species may be significant as a source of pathogenic endotoxins and allergens [15].

**Other bacteria.** Non-tuberculosis mycobacteria (*Mycobacterium gordonae*, *Mycobacterium flavescens*, *Mycobacterium chelonae*, *Mycobacterium fortuitum*, *Mycobacterium simiae*), which represent a hazard as a potential cause of infections in immunocompromised people, may occur in biofilms inside dental units [62]. In this environment, other Gram-positive bacteria were also found: *Streptococcus* spp., *Staphylococcus* spp., *Bacillus* spp. and *Actinomyces* spp. [6, 32, 51, 54].

## Fungi

### Fungi transmitted in saliva-droplet mode

Yeasts inhabiting the oral cavity, and especially species of *Candida* genus [34, 45, 62], are a potential risk for dentists. It is estimated that *Candida* occurs in the oral cavity of 25–50% of healthy people, and in the case of persons wearing dentures, the proportion increases to 60–100% [45]. This yeast is a common cause of stomatitis related to wearing dentures [45].

### Fungi transmitted in water-droplet mode

Yeasts, especially *Candida parapsilosis*, occur also in biofilms formed in dental unit waterlines. In the unit water, mould fungi of *Fusarium*, *Cladosporium*, *Alternaria*, *Penicillium* and *Scopulariopsis* can occur [62]. They represent a hazard for immunocompromised people in whom they may cause mycoses.

## Protozoa

### Protozoa transmitted in saliva-droplet mode

A protozoon *Pneumocystis carinii* can occur in the oral cavity and constitute a risk factor for immunocompromised people [34].

### Protozoa transmitted in water-droplet mode

In the biofilm of dental unit waterlines, free-living amoebae develop; the most common are: *Hartmannella* spp., *Vanella* spp. and *Vahlkampfia* spp. [5]. Equally, amoebae of *Naegleria* and *Acanthamoeba* genera occur there and may cause infections, especially in the case of compromised immunity.

## EPIDEMIOLOGICAL ASSESSMENT OF DENTISTS' OCCUPATIONAL HAZARDS AND THE BASIS FOR PREVENTION

### General assessment

From the review of microbiological hazards presented above, it follows that while performing procedures dentists are exposed to a vast range of harmful microbiological agents which are transmitted either from a patient through blood, direct contact or in the saliva-droplet mode, or they come from the water system and biofilm in dental units, and are transmitted in a water-droplet mode. The most serious risk factors are transmitted from a patient, especially through blood. In the majority of cases, these agents are well known and effective preventive measures are available. This is not the case of agents transmitted in a water-droplet mode which have been studied for a relatively short time, and the preventive measures are at the stage of testing.

### Assessment of risk factors transmitted from a patient through blood, direct contact and in saliva-droplet mode

Viruses, especially blood-borne HBV and HCV viruses, constitute the greatest hazard [34]. Blood-borne bacteria have not been reported as a serious risk factor, but some of saliva-borne bacteria, especially *Mycobacterium tuberculosis*, can be an important hazard [20].

#### Preventive measures

Preventive measures reducing the risk from microbiological factors transmitted from a patient include: personal protection means (masks, gloves) used by a dentist, disinfecting the patient's oral cavity and sterilization of dental instruments, the use of a rubber dam, sterilization of a workplace with UV radiation after a procedure, protective vaccination of dentists [34, 62]. Recently, American specialists, Palenik and Govoni [40], have strongly recommended immunization of dental personnel against the following diseases: hepatitis B, influenza, measles, parotitis, rubella, chicken pox and tuberculosis.

### Assessment of risk factors transmitted in water-droplet mode

Water in a dental unit is used to cool handpieces and patient's teeth. It may be delivered to a unit either from a municipal water system or from a special reservoir connected to a unit. Within a unit, water is distributed through a system of narrow plastic tubes in which there are conditions favouring the formation of biofilm consisting of bacteria, fungi and protozoa [53, 54]. Microbiological contamination of water in a unit may come from 3 sources: primary contamination of municipal water, a patient's saliva retracting through handpieces and unit waterlines, if there are no protective valves, biofilm developing in plastic tubing system in a dental unit [53].

Research conducted in North America has shown that the problem of microbiological, mainly bacterial, contamination of water in dental units is serious and requires comprehensive studies in order to adopt effective preventive measures. Williams *et al.* [61] found a high level of bacterial contamination of water from dental units in 150 dental clinics in the USA. Research conducted in 121 dental clinics in Canada brought similar results: high concentrations of bacteria, exceeding  $2 \times 10^5$  cfu/ml, were observed even in freshly installed units [6]. These values exceeded the norm of 200 cfu/ml, proposed by the American Dental Association (ADA) [42].

In the light of current knowledge, biofilm is the most important and most abundant source of microorganisms persisting in the water of dental units. Extensive growth of biofilm in dental unit waterlines was confirmed by numerous studies performed with the use of a scanning electron microscope [26, 47, 54].

According to Donlan's and Costerton's definition [14], biofilm is a sessile community of microorganisms that are irreversibly attached to a substrate and to each other, and embedded in the matrix of polymeric substances that they have produced, and exhibit an altered phenotype and genotype properties. As a whole, biofilm is a well-organized biostructure, compared to higher organisms tissues, characterised by an easy exchange of genetic material, easy absorption of nutrient substances from water, and high resistance to unfavourable physical and chemical conditions, including drying and application of disinfectants [54]. Isolating properties of the polysaccharide layer and the formation on the biofilm surface of a layer of cells specially adapted to decompose harmful substances, significantly enhance biofilm resistance to chemicals [11]. The thickness of the biofilm layer in dental unit waterlines equals 30-50  $\mu\text{m}$  [61].

#### Preventive measures

Chemical disinfectants removing biofilm are considered the most effective preventive measure [53]. However, biofilm is extremely resistant to these substances and in practice it is difficult to eliminate. Kettering *et al.* [22] applied 5 different disinfectants and achieved a decrease in biofilm to a level below the recommended ADA norm, but did not succeed in removing it completely. The research of numerous authors show that disinfection with products containing hydrogen peroxide, if applied regularly at weekly intervals is the most effective [51, 53, 58]. This was also confirmed in the research by Linger *et al.* [26] using scanning electron microscope. Disinfection with glutaraldehyde T4 also brings good results [36].

Other precautions against bacteriological contamination of water in dental units include installing special protective valves in handpieces to prevent patients' saliva penetrate the unit tubing [53].

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