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ORIGINAL ARTICLE

CONTENTS

Environmental microbial contamination as potential risk for healthcare associated infections after dental procedures: a multicenter study

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ABSTRACT

Aim: Aim this study to the microbial and molecular analysis of environmental (air, water and surfaces) microbial contamination in Ukrainian dental clinics.

Materials and Methods: This multicenter observational study was conducted between January 1, 2022, to December 31, 2024 in fifteen dental clinics located in five regions of Ukraine. Evaluation of the environmental contamination was performed according to microbial and molecular methods.

Results: During study period in dental clinics tap water, dental unit water systems, and water from the bottles attached to the dental chair units (before clinical activity and during clinical activities), and surfaces of dental equipment, and air (during clinical activities) was heavily contaminated with potential pathogens of healthcare associated infections (HAIs). The bacteria identified were mainly of the Gram-positive and Gram-negative species. The main potentially HAI-pathogenic of bacteria were *Escherichia coli* (13%), *Pseudomonas aeruginosa* (9.1%), *Enterococcus faecalis* (4%), *Klebsiella pneumoniae* (3.6%), *Acinetobacter lwoffii* (3.5%), *Acinetobacter baumannii* (3.3%), *Klebsiella oxytoca* (3.2%), *Serratia marcescens* (3.2%), *Stenotrophomonas maltophilia* (3.2%), *Staphylococcus aureus* (3.2%), *Enterococcus faecium* (3.1%), *Enterobacter cloacae* (3%), *Enterobacter aerogenes* (2.9%), *Burkholderia cepacia* (2.6%), *Providencia stuartii* (2.5%), *Proteus mirabilis* (2.2%), *Proteus rettgeri* (2.1%), and *Streptococcus pneumoniae* (2.1%).

Conclusions: During study period in dental clinics tap water, dental unit water systems, and water from the bottles attached to the dental chair units (before clinical activity and during clinical activities), and surfaces of dental equipment, and air (during clinical activities) was heavily contaminated with potential pathogens of HAIs. Patients are exposed during dental practice to an infective risk.

KEY WORDS: dental clinics, environmental contamination, microorganisms, infective risk, dental unit water systems, dental equipment surfaces, Ukraine

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INTRODUCTION

Patients and healthcare workers are exposed during dental practice to an infective risk, which derives from microorganism suspended in aerosols. Therefore, environmental microbiological monitoring is crucial to control in dental settings and represents a good instrument to detect critical situations [1, 2].

Monitoring of microbial contamination in the air, water and surfaces has been carried out for over 100 years and its importance has been widely pointed out, and more recently the use of molecular genetic tools has been implemented [3]. According to the literature, research interests include various fields from the environmental point and human health perspective [4] due to pathogens such as bacteria, fungi and viruses. However, up to now there are no established international protocols or standards for detecting diversity of these microorganisms.

According to the literature, research interests include various fields from the environmental point and human health perspective due to pathogens such as bacteria, fungi and viruses. Recent publications have showed the possibility to collect microbial DNA through air [3, 5], water [6, 7] and surfaces [8] sampling, providing insight into microorganism diversity. Given its health implications, a primary focus of studies is the analysis of microbiological contamination in the air, water and surfaces in healthcare setting. Modern epidemiological studies from various countries indicate that currently the most hospitals have high level of bacterial contamination of surfaces.

Most epidemiological studies from various countries indicate that currently the most hospitals have high level of bacterial contamination of surfaces, with even higher rates observed in dental clinics. One of the leading causes of worldwide healthcare-associated infections (HAIs) in dental clinics are bacteria. Some of these pathogens are transmitted through the air and are opportunistic while others are host specific. Monitoring is therefore of importance in programs mitigating further spread of HAIs. However, monitoring airborne fungi and bacteria can be very challenging, as many are very difficult to identify accurately. Presently only using DNA analysis makes it possible to accurately distinguish different opportunistic microorganisms' species in air, water and surfaces samples.

Currently, in Ukraine the microbiological contamination of the environment in dental healthcare facilities was poorly studied. A previous study has focused on the prevalence of HAIs and antimicrobial resistance of the responsible pathogens [9] and nosocomial transmission of multi-drug-resistant organisms in Ukrainian hospitals [10], and on the evaluation of bacterial contamination in the inanimate environment surfaces in acute care hospitals [11]. However, these results do not provide sufficient information about the factors contributing to the spread of the incidence of HAIs in Ukrainian dental clinics, which requires further study.

AIM

The aim of this study was to the microbial and molecular analysis of environmental (air, water and surfaces) microbial contamination in Ukrainian dental clinics.

MATERIALS AND METHODS

STUDY DESIGN AND SETTING

This multicenter observational study was conducted between January 1, 2022, to December 31, 2024 in fifteen dental clinics located in five regions (Lviv, Kharkiv, Kyiv, Zhytomyr, Odessa) of Ukraine. The clinics were recruited on a voluntary basis. All dental chair and ancillary equipment in these clinics are used for routine general oral healthcare procedures. These procedures include oral examinations, dental extractions, endodontics, prosthodontic procedures, restorations and prophylaxis among others. In dental clinics water is sourced from the mains municipal supply, and stored in secondary reservoirs such as large tanks from where water is then supplied to dental unit water systems (DUWLs). All dental clinics used a closed system, where the reservoir bottles were filled with distilled water and attached to the dental chair units. In all dental clinics surfaces and the handpieces are routinely treated with disinfectants between patients or are autoclaved. Wet cleaning was carried out once a day - after the occupancy period in all dental offices. Concerning ventilation, 90% of offices had an air-conditioning system, and 95% had mechanical ventilation. The ventilation of the operating areas for at least 10–15 min was implemented between each patient. All dental operating areas, from the least critical to the most critical, were adequately cleaned and disinfected. Inclusion criteria: all dental clinics willing to partake in the study and had to be perform routine dental procedures. Exclusion criteria: dental clinics that did not sign a consent letter to participate in this study.

SAMPLING STRATEGIES

Microbial contamination of air, water, and environmental surfaces, was assessed in each dental clinic. Microbial contamination was evaluated for the following types of dental treatments: (a) orthodontics, (b) periodontic, (c) oral surgery, and (d) prosthodontic. Samples were collected in the closed dental operatory. In this study, water and surfaces were sampled before clinical activity and after clinical activity. Air was sampled 5 min before starting clinical activity, during, and immediately after the clinical activity. Evaluation of the microbial contamination of water, air and surfaces was performed according to previously reported methods. All samples were transported to the Zarifa Aliyeva International Center of Medical Science Laboratory (Ukraine) and processed (for microbial and molecular analysis). Evaluation of the microbial contamination of water, air and surfaces was performed according to microbial and molecular methods, which are briefly described below.

MICROBIAL ANALYSIS

AIR SAMPLING

In this study, microbial contamination in air was evaluated through active sampling, to measure the concentration of microorganisms in the air, and by passive sampling, to measure the rate at which viable particles settle on surfaces. Microbial contamination in air was evaluated by DSTU ISO 14698-1:2008 [12]. TVC was performed both for active and passive sampling using Slit-to-Agar biological air sampler. The air sampler was placed at the average working distance of the clinicians involved in the study (and from the aerosol source), 30 cm, at the height of 1.5 m above the floor, which corresponded to the area where the patient was breathing. Passive sampling was performed to determine the Index of Microbial Air Contamination (IMA) who corresponds to the number of CFU counted on a Petri dish with a diameter of 9 cm placed according to 1 m above the floor, about 1 m away from dental chair units or the patient's mouth for 1 h. After incubated period all colonies were counted using the ScanStation Automatic colony counter (interscience) and reported as colony forming units. The total numbers of colony-forming units (CFU) were determined, and the data were expressed as the number of CFU per cubic meter of air sampled. Passive sampling was performed to determine the Index of Microbial Air Contamination (IMA) Microbial species identification was performed with standard microbial methods.

WATER SAMPLES

Tap water, DUWS water, and distilled water from the reservoir bottles attached to the dental chair units were sampled to determine the total viable count (TVC), and the presence of *Legionella* spp. *Pseudomonas aeruginosa, Escherichia coli*, intestinal enterococci, and other microorganisms. In this study for DUWS, the TVC was measured on every handpiece (ablator, air–water syringe, cup filler, microengine, turbine). The detection of the bacteria was performed according to the international standards for water quality: E DSTU EN ISO 6222:2002 for total viable count [13], DSTU EN ISO 11731:2022 for *Legionella* [14], EN ISO 16266 for *P. aeruginosa* [15], DSTU EN ISO 9308-1:2022 for *Escherichia coli* and coliform bacteria [16], and DSTU EN ISO 7899-2:2022 for intestinal enterococci [17].

SURFACE SAMPLES

Six surface samples were collected in each dental operatory. Flat surfaces were sampled by agar contact plates through an applicator. Non-flat surfaces were sampled using swabs with tubes, which contains a disinfectant inhibitor. All petri dishes were incubated for bacteria and fungi. The results were expressed as CFU/cm^2. Bacteria and fungi were isolated and identified using aVitek-2 automated system.

MOLECULAR ANALYSIS

Genomic DNA was extracted from a single colony of each isolate using QIAmp® DNA Mini kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The quality of DNA extraction was measured by agarose gel electrophoresis. In this study DNA was quantified by ultraviolet spectrophotometer. Extracted genomic DNA was amplified using a set of primers (341F: CCTACGGGNGGCWGCAG; 806R: GGACTACHVGGGTATCTAAT) targeting bacterial 16SrRNA genes [18]. Primers (ITS3_KYO2: GATGAAGAACGYAGYRAA; ITS4: TCCTCCGCTTATTGATATGC) were used for the fungal ITS2 gene amplification [19]. The purified amplification products were pooled and sequenced on an Illumina platform following the manufacturer's recommendations.

ETHICS

The Ethics Board of the Ukrainian Association of Infection Control and Antimicrobial Resistance has approved the protocols of this study. All dental clinics and patients in the study signed a letter of consent.

STATISTICAL ANALYSIS

Microbiological data were collected in Microsoft® Excel (Microsoft Corporation, Redmond, WA, USA). Descriptive statistical analysis was performed to provide mean, standard deviation, 95% confidence interval. In this study all the data are presented as numbers and percentages. The microbial contamination was summarised using descriptive statistics separately by type of pathogen and location. Statistical significance in this study was assumed for p values lower than 0.05.

RESULTS

MICROBIAL ANALYSIS

Water contamination. A total of 2,520 water samples were collected, of which 76,7% (95% CI: 75.9-77.5) samples did not meet microbial parameters to Ukrainian National Standard DSTU 7525:2014 (2015) and the State Sanitary Norms and Rules of the DSanPiN 2.2.4-171-10, section Indicators of Epidemiological Safety of Drinking Water (TVC<100 CFU/mL). During study period (2020-2022) the share of non-standard TVC for tap water, DUWSs, and water from the reservoir bottles attached to the dental chair units was 62.5% (224/360), 87.4 % (1,573/1,800), and 38.1% (137/360), respectively. In 2022, compared to the 2020 for the tap and DUWSs water quality there was an increase in the number of non-standard water samples for TVC indicators. In the largest specific weight of non-standard (on TVC) water samples was detected in Kyiv, Zhytomyr and Kharkiv region of Ukraine.

The TVC values for tap water and DUWSs, varied markedly between the 15 dental clinics, and significantly decreased between before clinical activity and after clinical activity ($p < 0.0012$). The TVC median values for

Table 1. Distribution of main microorganisms (>1%) isolated from water samples in Ukrainian dental clinics (2020-2022)

	All		Source of water samples					
Microorganism	**isolates**		**Tap**		**DUWS**		**Bottle**	
	(n=3,176)		**(n=965)**		**(n=1,295)**		**(n=595)**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Gram-positive cocci								
Enterococcus faecalis	394	12.4	153	15.9	184	14.2	57	9.6
Enterococcus faecium	311	9.8	128	13.3	139	10.7	44	7.4
Staphylococcus aureus	96	3.0	-	-	51	3.9	45	7.6
Staphylococcus haemolyticus	36	1.1	14	1.5	15	1.2	7	1.2
Streptococcus pneumoniae	53	1.7	13	1.3	28	2.2	12	2.0
Gram-negative bacilli								
Acinetobacter lwoffii	107	3.4	39	4.0	47	3.6	21	3.5
Acinetobacter baumannii	92	2.9	0	0	54	4.2	38	6.4
Burkholderia cepacia	117	3.7	36	3.7	57	4.4	24	4.0
Enterobacter cloacae	124	3.9	42	4.4	51	3.9	31	5.2
Enterobacter aerogenes	121	3.8	43	4.5	51	3.9	27	4.5
Escherichia coli	491	15.5	189	19.6	221	17.1	81	13.6
Klebsiella pneumonia	99	3.1	30	3.1	48	3.7	21	3.5
Klebsiella oxytoca	112	3.5	35	3.6	48	3.7	29	4.9
Providencia stuartii	88	2.8	29	3.0	35	2.7	24	4.0
Pseudomonas aeruginosa	372	11.7	131	13.6	166	12.8	75	12.6
Stenotrophomonas maltophilia	117	3.7	39	4.0	47	3.6	31	5.2
Serratia marcescens	125	3.9	44	4.6	53	4.1	28	4.7

Source: compiled by the authors of this study

tap water collected pre-clinical activity ranged from 3.6×10^4 CFU/mL to 7.8×10^4 CFU/mL. While at the end of the clinical activity the corresponding median values ranged from $3,8 \times 10^3$ CFU/mL to 6.8×10^3 CFU/mL. The TVC median values for DUWSs pre-activity ranged from 12.6×10^4 CFU/mL to 19.8×10^4 CFU/mL. Post-activity median values ranged from 3.9×10^4 CFU/mL to 9.7×10^4 CFU/mL. Of the 1,800 handpieces sampled, the cup filler had the lowest median contamination values before (3.1×10^3 CFU/mL) and after (2.2×10^3 CFU/mL) clinical activity. The air–water syringe and ablator showed the highest microbial contamination before clinical activity (TVC was 8.7×10^5 CFU/mL and 5.3×10^5 CFU/mL, respectively). While the turbine had the highest contamination values after clinical activity (3.1×10^5 CFU/mL).

A total of 3,176 isolates were detected from 2,520 water samples. Among the isolates, gram-negative bacteria and gram-positive cocci was 85.8% and 12.1%, respectively. Fungi was 2.1%. The main microorganisms isolated from water samples are shown in Table 1.

Air contamination. A total of 1,080 air samples were collected in dental clinics. When the air samples were analyzed at before the start of the dental procedure, a low number of bacterial colonies was detected. The species identified in dental clinics were mainly Gram-positive, including *Staphylococcus* sp. (45–85 CFU/m^3), *Bacillus* spp., and fungi (10–20 CFU/m^3). For the microbial contamination in the air, active and passive sampling median values showed a significant increase between before clinical activity and during dental treatments ($p<0.0014$), followed a decrease once the clinical activity was over ($p<0.0012$). During clinical activity median values ranged, for in dental clinics, from 396 CFU/m^3 to 2,735 CFU/m^3. No *Legionella* spp. were isolated in any Ukrainian dental clinic. The bacteria identified were mainly of the Gram-positive species and included *Staphylococcus* sp. and *Bacillus* sp. with a greater number of colonies. The most abundant species belonged to the *Staphylococcus* sp. (*S. aureus, S. epidermidis,* and *S. haemolyticus*) and *Streptococcus* sp. family, including *Streptococcus salivarius* and *Streptococcus mutans*, among the main species in the oral cavity. In this study there was a significant correlation between active

Table 2. Distribution of microorganisms isolated from environmental samples in Ukrainian dental clinics (2020-2022)

Microorganism	**All isolates (n=17,482)**		**Water**				**Air**				**Surfaces**			
			BCP (n=4,223)		**ACP (n=1,384)**		**BCP (n=124)**		**ACP (n=987)**		**BCP (n=1,286)**		**ACP (n=9,478)**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Gram-positive cocci														
Enterococcus hirae	73	0.4	5	0.1	2	0.14	-	-	2	0.2	32	2.49	32	0.4
Enterococcus faecalis	703	4.0	208	5.2	71	5.13	-	-	38	3.85	38	2.95	348	3.7
Enterococcus faecium	549	3.1	127	3.2	41	2.96	-	-	35	3.55	35	2.72	311	3.3
Kocuria varians/rosea	85	0.5	7	0.2	2	0.14	-	-	1	0.1	37	2.88	38	0.4
Micrococcus sp.	152	0.9	16	0.4	3	0.22	6	4.8	12	1.22	52	4.04	63	0.7
Staphylococcus aureus	553	3.2	71	1.8	9	0.65	7	5.6	59	5.98	49	3.81	358	3.8
Staphylococcus capitis	77	0.4	29	0.7	4	0.29	4	3.2	4	0.41	14	1.09	22	0.2
Staphylococcus epidermidis	486	2.8	29	0.7	3	0.22	5	4.1	19	1.93	19	1.48	411	4.4
Staphylococcus haemolyticus	208	1.2	44	1.1	13	0.94	7	5.6	33	3.34	33	2.57	78	0.8
Staphylococcus hominis	143	0.8	33	0.8	9	0.65	12	9.7	28	2.84	28	2.18	33	0.4
Staphylococcus sciuri	55	0.3	11	0.3	2	0.14	2	1.6	4	0.41	18	1.4	18	0.2
Strptococcus viridans	200	1.1	5	0.1	7	0.51	4	3.2	4	0.41	9	0.7	171	1.8
Streptococcus pneumoniae	360	2.1	76	1.9	25	1.81	9	7.3	9	1.91	13	1.01	228	2.4
Streptococcus pyogenes	316	1.8	66	1.7	20	1.45	5	4.1	5	0.51	11	0.86	209	2.2
Streptococcus sp.	105	0.6	49	1.2	7	0.51	4	3.3	4	0.41	12	0.93	29	0.3
Other Gram-positive cocci	40	0.2	11	0.3	3	0.22	3	2.4	3	0.3	5	0.39	15	0.2
Gram-negative bacilli														
Acinetobacter lwoffii	612	3.5	122	3.1	42	3.03	4	3.3	28	2.84	18	1.4	398	4.2
Acinetobacter baumannii	574	3.3	114	2.9	39	2.82	5	4,1	26	2.63	19	1.48	371	3.9
Acinetobacter sp.	81	0.5	44	1.1	15	1.08	1	0.8	3	0.3	9	0.7	9	0.1
Bacillus cereus	127	0.7	32	0.8	10	0.72	4	3.3	5	0.51	18	1.4	58	0.6
Bacillus sp.	85	0.5	24	0.6	7	0.51	3	2.4	6	0.61	11	0.61	34	0.4
Burkholderia cepacia	453	2.6	144	3.6	49	3.54	-	-	33	3.34	33	2.57	194	2.1
Enterobacter cloacae	522	3.0	137	3.4	47	3.4	1	0.8	34	3.44	34	2.64	269	2.8
Enterobacter aerogenes	506	2.9	124	3.1	43	3.11	-	-	31	3.14	31	2.41	277	2.9
Enterobacter sp.	253	1.5	22	0.6	10	0.72	-	-	7	0.71	17	1.32	197	2.1
Escherichia coli	2,266	13.0	812	20.4	281	20.3	-	-	162	16.4	22	1.71	989	10.4
Klebsiella pneumonia	631	3.6	196	4.9	69	4.99	3	2.4	41	4.15	11	0.86	311	3.3
Klebsiella oxytoca	565	3.2	161	4.1	56	4.05	2	1.6	37	3.75	12	0.93	297	3.3
Legionella pneumophila	19	0.1	11	0.3	4	0.29	2	1.6	2	0.2	-	-	-	-
Moraxella sp.	278	1.6	43	1.1	14	1.01	1	0.8	9	0.91	19	1.48	192	2.1
Providencia stuartii	435	2.5	125	3.1	44	3.18	2	1.6	28	2.84	19	1.48	217	2.3
Proteus mirabilis	384	2.2	41	1.1	27	1.95	-	-	7	0.71	18	1.4	291	3.1
Proteus rettgeri	373	2.1	46	1.2	25	1.81	-	-	9	0.91	17	1.32	276	2.9
Pseudomonas aeruginosa	1,596	9.1	501	12.6	155	11.2	-	-	121	12.7	21	1.63	798	8.4
Pseudomonas luteola	69	0.4	12	0.3	4	0.29	-	-	-	-	28	2.18	25	0.3
Pseudomonas sp.	58	0.3	19	0.5	6	0.43	-	-	3	0.3	14	1.09	16	0.2
Stenotrophomonas maltophilia	558	3.2	144	3.6	50	3.61	-	-	31	3.14	6	0.47	327	3.5
Serratia marcescens	561	3.2	161	4.1	56	4.05	-	-	38	3.85	9	0.7	297	3.1
Other Gram-negative bacilli	41	0.2	9	0.3	3	0.22	3	2.4	2	0.2	12	0.93	12	0.1
Fungi														
Aspergillus flavus	88	0.5	19	0.5	5	0.36	3	2.4	3	0.3	19	1.48	39	0.41
Aspergillus niger	73	0.42	22	0.6	4	0.29	4	3.3	3	0.3	15	1.17	25	0.26
Aspergillus ochraceus	45	0.26	9	0.2	-	-	-	-	-	-	13	1.01	23	0.24
Candida albicans	347	1.98	72	1.8	25	1.81	1	1.8	12	1.22	18	1.4	219	2.31
Candida glabrata	344	1.97	58	1.46	20	1.45	-	-	11	1.11	21	1.63	234	2.47
Candida krusei	290	1.66	43	1.08	15	1.08	1	0.8	1	0.1	19	1.48	211	2.23
Penicillium sp.	91	0.52	19	0.48	1	0.07	1	0.8	3	0.3	19	1.48	48	0.51
Other fungi	40	0.23	5	0.13	3	0.22	2	1.6	2	0.2	14	1.09	14	0.15

BCP, before clinical practice; *ACP*, after clinical practice

Source: compiled by the authors of this study

Table 3. Distribution of main potential human pathogenic microorganisms(n=17,483) isolated from environmental samples of Ukrainian dental clinics (2020-2022)

Microorganisms	n	%	95% CI
Escherichia coli	2,266	13.0	12.71 – 13.21
Pseudomonas aeruginosa	1,596	9.13	8.91 – 9.35
Enterococcus faecalis	703	4.02	3.87 – 4.17
Klebsiella pneumonia	631	3.61	3.47 – 3.75
Acinetobacter lwoffii	612	3.5	3.36 – 3.64
Acinetobacter baumannii	574	3.28	3.15 – 3.41
Klebsiella oxytoca	565	3.23	3.1 – 3.36
Serratia marcescens	561	3.21	3.08 – 3.34
Stenotrophomonas maltophilia	558	3.19	3.06 – 3.32
Staphylococcus aureus	553	3.16	3.03 – 3.29
Enterococcus faecium	549	3.14	3.01 – 3.27
Enterobacter cloacae	522	3.0	2.86 – 3.12
Enterobacter aerogenes	506	2.89	2.76 – 3.02
Staphylococcus epidermidis	486	2.78	2.66 – 2.9
Burkholderia cepacia	453	2.59	2.47 – 2.71
Providencia stuartii	435	2.49	2.37 – 2.61
Proteus rettgeri	373	2,13	2.02 – 2.24
Proteus mirabilis	384	2.2	2.09 – 2.31
Streptococcus pneumoniae	360	2.06	1.95 -2.17
Candida albicans	347	1.98	1.87 – 2.09
Candida glabrata	344	1,97	1.86 – 2.08
Streptococcus pyogenes	316	1.81	1.71 – 1.91
Candida krusei	290	1.66	1.56 – 1.76
Moraxella sp.	278	1.59	1.5 – 1.68
Enterobacter sp.	253	1.45	1.36 – 1.54
Staphylococcus haemolyticus	208	1.19	1.11 – 1.27
Strptococcus viridans	200	1.14	1.06 – 1.22

Source: compiled by the authors of this study

and passive air sampling (p<0.0013). The air samples collected after the clinical activity showed a decrease in bacterial load compared to the previous sampling.

Contamination of surfaces. During study period 2,160 samples were collected from six dental equipment surfaces (light, dental spittoon, countertop, head board, chair arm rest, and air/water syringe) of the 15 dental clinics. The dental equipment surfaces were chosen due to their frequent interaction with the patient or the dental doctor, being the most likely surfaces to accumulate bacteria. Bacteria and fungi isolated from samples were cultured, then counted and identified. There was no statistical difference in microbiological contamination between dental clinics. In dental equipment surfaces before the start of the dental procedure was a poor microbiological contamination with rare pathogenic microorganisms. The only significant difference was a higher fungal contamination of surfaces in prosthodontic dental rooms compared with oral surgery, periodontic, and orthodontics rooms (p < 0.03). Oral surgery, periodontic, and orthodontics offices with daily wet cleaning had surfaces significantly less contaminated by fungi but similarly contaminated by bacteria (p < 0.002). The most prevalent microorganisms in oral surgery, orthodontics, periodontic, and prosthodontic surface samples before clinical activity were Gram-positive cocci, mainly *Micrococcus, Staphylococcus*, and *Kocuria*, followed by filamentous fungi, mainly *Cladosporium* and *Penicillium*; Gram-negative bacilli, mainly endospore-forming Gram-positive bacilli with *Bacillus*. The surfaces samples collected after the clinical activity showed a significant increase in bacterial load compared to the previous sampling (p<0.001). On the surfaces, concentrations of bacteria and fungi were from 6,287 to 29,756 CFU/100 cm^2 and from 136 to 778 CFU/100 cm^2, respectively. During study period in dental equipment surfaces samples was detected 27 bacterial species from 8 genera, and 10 filamentous fungal species from 5 genera. Species of *S. aureus, S. epidermidis, E. faecalis, E. faecium, A. lwoffii, B. cepacia, E. cloacae, E. aerogenes, E. coli, K. oxytoca, P. aeruginosa, S. maltophilia*, and *S. marcescens* were found after clinical activity in all orthodontics, periodontic, oral surgery, prosthodontic room. The highest microbial diversities were found in surfaces of air/water syringe and light (orthodontics, periodontic, oral surgery, prosthodontic room) for bacteria and in the prosthodontic rooms for fungi and yeasts.

MOLECULAR ANALYSIS

In this study the 16S rDNA gene sequencing showed that the bacterial communities of all samples (air, water and environmental surfaces) covered 29 classes, 61 orders, 122 families, 143 genera, and 411 species. The distribution of microorganisms isolated from environmental samples in Ukrainian dental clinics in Ukraine is shown in table 2. The highest number of microorganisms was found in water samples and dental equipment surfaces. Potential human pathogens were detected in water, air and surface samples, including 7 genera of bacteria and 5 genera of fungi. The main potentially human-pathogenic genera of bacteria with relative abundance over 1% were *E. coli* (13%), *P. aeruginosa* (9.1%), *E. faecalis* (4%), *K. pneumoniae* (3.6%), *A. lwoffii* (3.5%), *A. baumannii* (3.3%), *K. oxytoca* (3.2%), *S. marcescens* (3.2%), *S. maltophilia* (3.2%), *S. aureus* (3.2%), *E. faecium* (3.1%), *E. cloacae* (3%), *E. aerogenes* (2.9%), *B. cepacia* (2.6%), *P. stuartii* (2.5%), *P. mirabilis* (2.2%), *P. rettgeri* (2.1%), *S. pneumoniae* (2.1%), *C. albicans* (2%), and other microorganisms (Table 3).

DISCUSSION

The environmental microbial contamination in Ukrainian dental setting was never studied. Therefore, the present study aimed to assess microbial contamination in the dental environment, identify the microorganisms involved, and determine their count in dental clinics. To the authors' knowledge, this study is the first to microbial and molecular analysis the microbiological contamination of air, water and dental equipment surface in dental setting. Environmental microbial contamination in dental clinics was comprehensively evaluated, with more abundance of bacterial communities using standard microbial methods and high-throughput sequencing technology. The findings of this study revealed tap water, DUWLs, and water from the bottles attached to the dental chair units (before clinical activity), and surfaces of dental equipment, and air (during clinical activities) are heavily contaminated with potential human pathogens. These results are consistent with previous studies [8, 20, 21]. Our previous study showed that the microbial load in dental rooms air is highly influenced by the ventilation and number of patients and health care workers, who can be a potential source of microorganisms as they shed the microorganisms from the skin squames and the respiratory tract [22].

The 16S rDNA gene sequencing showed that the main potentially human-pathogenic species of bacteria with relative abundance over 3% were *E. coli* (13%), *P. aeruginosa* (9.1%), *E. faecalis* (4%), *K. pneumoniae* (3.6%), *A. lwoffii* (3.5%), *A. baumannii* (3.3%), *K. oxytoca* (3.2%), S. marcescens (3.2%), *S. maltophilia* (3.2%), *S. aureus* (3.2%), *E. faecium* (3.1%), and *E. cloacae* (3%). The highest numbers of these pathogens were found in DUWLs water. There was noticeable contamination of air and dental equipment in surfaces the active dental treatment area. The bacterial aerosols were able to spread into areas where there was dental activity.

The present study showed that *P. aeruginosa*, *E. coli*, and *Enterococcus* sp. is the most important and difficult to eliminate pathogens commonly found in DUWLs. These pathogens originated from DUWLs may generate healthcare-associated infections (HAIs) [9], including like dental abscesses among patients [23]. The results this study highlights the need to perform microbiological analysis of DUWLs water routinely. However, to date, there is no standard about microbial water quality control of DUWL systems in dental healthcare setting. The water quality monitoring by sample analysis is recommended in the USA in dental setting to detect of pathogenic bacteria [24, 25]. In a previous study showed that P. aeruginosa could survive in DUWLs despite a disinfection, and the resistance of this strains to several biocides has been confirmed by antimicrobial susceptibility tests [26].

According to the literature, various regulations have been formulated to control clinical DUWLs contamination. The American Dental Association (ADA) set a limit of ≤ 200 CFU/mL on the TVC in the output water of the dental unit. In some EU countries and China, the drinking water standard of < 100 CFU/ml is referenced, clarified in the standard for prevention and control of HAIs in dental setting that the water from DUWLs should meet the drinking water standard (< 100 CFU/ml) [27]. In Ukraine there are no legislated parameters relating to the presence of P. *aeruginosa, E. coli, Enterococcus* sp., and other bacterial species in water from DUWLs.

According to the Ukrainian State Sanitary Norms and Rules of the DSanPiN 2.2.4-171-10, these bacterial species should not be listed to national statistical reports under water quality. In Ukraine there are two standards at the same time: SanPiN 2.2.4-171-10 and State standards of Ukraine DSTU 7525: 2014 (Drinking water). Currently, SanPiN 2.2.4-171-10 is a binding normative legal act. However, National standards of Ukraine DSTU 7525:2014 (2015) are optional. The Ministry of Health of Ukraine currently has no explicit requirements for the microbial parameters of water, which is supplied to DUWLs. Also, not any regulations related to aspects to curb the transmission of HAI or for infection control issues related to the dental setting.

STRENGTHS AND LIMITATION

This study is the first to environmental microbial contamination in dental clinics was comprehensively evaluated, with more abundance of bacterial communities using standard microbial methods and high-throughput sequencing technology. A limitation of this study is the absence of microbiological analysis the disinfection to evaluate the efficiency of the disinfectants used. The quantity of bacteria detected in water and dental equipment surfaces significantly. Further studies are required to assess the contamination rate as risk factor of HAIs and mechanisms of antimicrobial resistance these pathogens. In addition, further studies should integrate data on microbial water, air and environmental surfaces contamination with microbiologically examined pathogens and oropharyngeal swabs from patients with dental HAIs to identify pathogens from the oral cavity and perform the appropriate patient-related risk assessment for airborne and aerosol-transmitted infections in a dental setting.

CONCLUSIONS

The findings of this study revealed during study period in dental clinics tap water, DUWLs, and water from the bottles attached to the dental chair units (before clinical activity and during clinical activities), and surfaces of dental equipment, and air (during clinical activities) was heavily contaminated with potential pathogens of HAIs. Patients are exposed during dental practice to an infective risk. The primary sources of environmental contamination in Ukrainian dental clinics are bacteria from DUWLs water. The water output microbial contamination from DUWLs depends on the feed water of the supply unit, which are the main municipal water supply, external storage tanks, or isolated bottled reservoirs. The bacterial aerosols were able to spread into areas where there was dental activity. Therefore, environmental microbiological monitoring is crucial to infection control in dental settings. To control the environmental contamination, an internal control plan in each dental clinic is necessary, including an water and dental equipment surfaces disinfection a followed by a microbiological analysis to control its efficiency. Microbiological monitoring of DUWLs water should be performed routinely to control the microbial contamination of water used for dental care and to prevent of HAIs. This is necessary not only to assess the efficiency of preventive measures but also to guide the implementation of corrective strategies.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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