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Original Research Article.

Evaluation of Microbial Air Contamination in Dental Surgery Clinics

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ABSTRACT

Background: In dental surgery clinic (DSC) environment the dentistry professionals and patients are daily exposed to a great variety of infectious agents which transported by aerosols and droplets or settle on environmental surfaces, produced during dental practice.

Objective and Method: The aim of this study was to assess the air microbial contamination quality and quantity during manned and non-manned conditions in DSCs by using exposure and air sampling plate techniques, also investigating the antibiotic susceptibility to pathogenic microbial isolates by using the vitek 2 system.

Results and Conclusion: The mean values of bacterial and fungal CFUs in the multi DSCs were 4.9 CFU/hr and 1 CFU/m^3 during the manned conditions while in non-manned conditions was 0.4 CFU/hr and 0.28 CFU/m^3 when used exposure and air sampling plates techniques respectively. The predominant bacterial isolates from DSCs were commensal and no risk found to healthcare staff and patients, which includes Micrococcus luteus, Micrococcus lylae, Staphylococcus haemolyticus, Staphylococcus lugdunensis, Bacillus sp and Pseudomonas stutzeri around 26–23, 20–22, 18–20, 9.5–12, 22–24 and 0–0.25% respectively, while the fungal isolates are Penicillium sp and Aspergillus sp were 0–0.25 and 0.25% respectively in the DSCs. Gentamicin, Levofloxacin, Tigecycline, Tobramycin and Trimethoprim/sulfamethoxazole, were given sensitive antibiotics for M. luteus, M. lylae, S. haemolyticus, S. lugdunensis and P. stutzeri. While the Batrafen, Canasten, Flucoral and Mycosat antifungal agents were given a sensitive antifungal for Penicillium sp and Aspergillus sp. In the present research the microbial air count of the DSCs was significantly higher in manned than non-manned conditions ($p < 0.001$). Although, the microbial counts in DSCs during the manned and non-manned conditions were presented with the limits according to the European Union standard for the air system.

Keywords: Dental Surgery Unit, Air Contamination, Antimicrobial Resistant, Exposure Plate, Air Sampling Technique.

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INTRODUCTION

Spread of infection in the dental clinic's environment is of major concern to the dental community mainly because it carries a possible risk of transmission of infectious agents and their potential effects on the health of the dental personnel and the individuals.[1] In dental practice, workers staff, visitors and patients can be exposed to pathogenic bacteria such as *Staphylococcus* sp, *Streptococcus* sp and *M. tuberculosis* by transmitted through indirect contact with contaminated instruments or settle on environmental surfaces and mucosal contact with droplets generated by coughing or sneezing or inhalation of airborne microorganisms or direct contact with blood or other oral fluids.[2,3]

The airborne microorganisms were generated by dental procedures such as high-speed dental drill, ultrasonic scaler and water/air syringe usually generate fine microbial aerosols 50 µm or less in diameter derived from blood, saliva, tooth debris, dental plaque, calculus, and restorative materials and these fine aerosols, that survive for a long time and become a source of potential infection, unless they are eliminated by disinfection procedures. Through this kind of health care practice, there is increasing evidence that use of dental equipment has been shown to responsible for the production of hazardous aerosols.[4-7]

Bioaerosols were generated during dental treatment or surgery can be defined as particles <50 µm in diameter containing microorganisms in saliva, blood, plaque and nasopharyngeal secretions that are small enough to stay airborne for an extended period of time before they enter the respiratory tract or settle down on the environmental surface and represents a potential mechanism for the spread of infection.[8,9]

Dental procedures in the dental surgery environment can generate large quantities of <3 μm aerosol particles which contaminated with bacteria and fungi from the oral cavity (from saliva and dental biofilms), as well as viruses from the patient's blood. Transmission of infection can occur through the airborne inhalation route, contaminated hands with respiratory droplets and contact with infective agents present in either the droplets or aerosol particles from saliva and respiratory fluids.[10-12]

Therefore, this study aimed to determine the degree of air bacterial and fungal contamination of dental surgery clinics with respect to acceptable microbial load standards and measure antimicrobial susceptibility pattern of the pathogenic isolates.

MATERIALS AND METHODS

Sampling Sites: This research measures the air quality and quantity in the Dental Teaching Hospital, Umm Al-Qura University in Makkah City, Saudi Arabia. Samples were collected from 15 DSCs. The exposure and air sampling plates were done during manned and non-manned conditions. All samples were distributed in the DSCs and all data was recorded during the experiments as shown in DSCs layout Figure 1.

Evaluation of Viable Air Contamination

a: Settle Plate Methods

The settle plate method was carried out by using 90 mm tryptic soy agar (TSA) plates medium in four locations (a) on the tray surface, (b) on the table, (c) front the dental chair, (d) beside the doctor stool, into the DSCs according to Pasquarella et al., 2000. All data was recorded and compared with the European Union standard for the air system.[13]

b: Air Sampling Methods

The air samples were collected in the DSC from three locations (a) on the tray surface, (b) on the table, (c) front the dental chair, according to Lembke et al., 1981. All data was recorded and compared with the European Union standard for the air system.[14]

Figure 1: Exposure plates and air sampling distribution into DSC. (a) on the tray surface, (b) on the table, (c) front the dental chair, (d) beside the doctor stool. [Exposure plate site: a,b,c,d; Air sampling plate site: a,b,c]

Bacterial and Fungal Identification

The bacterial isolates were identified by morphological, biochemical characteristics by VITEK 2 system. While the fungal isolates were examined microscopically and identified by morphology, spore and hyphal characteristics and microscopic appearance according to Leck, 1999.[15]

Susceptibility to Antibiotics

The antibiotic susceptibility of pathogenic bacterial isolates determined by using the VITEK 2 system and evaluated according to the Clinical Laboratory Standards Institute (CLSI) guideline 3rd edition (Jean et al., 2016), while the antifungal susceptibility test of fungal isolates determined by agar disk diffusion method according to El banna et al., 2014.[16,17]

The following antifungal were included in the study: Batrafen, Canasten, Flucoral, Fungican and Mycosat against fungal isolates. All tests were performed in Mueller–Hinton Agar (Oxoid, UK) in triplicate and classified as susceptible or resistant.

Statistical Analysis: All determinations were performed in triplicate. The results are reported as the mean values, SD and 95% confidence interval of CFU were calculated. Due to the normal distribution of data, their mean used a statistical descriptor. The paired *t*-test was used for analyzing the difference between microbial counts during manned and non-manned conditions. Statistical significance was assumed for *p* values lower than 0.001. Statistical analysis was carried out using portable SPSS statistics version 19.

Ethical Disclosures: The authors announce that no experiments were performed on voluntaries or animals and no data were collected from patient in this research. The authors have obtained the written approval of the Teaching Dental Hospital, Umm Al-Qura University, Makkah, Saudi Arabia to do this study.

RESULTS AND DISCUSSION

The results of this study showed that aerosols settle on environmental surfaces and may act as an important source of infection in DSC during the manned and non-manned conditions, investigated the microbial contamination levels, detected the bacterial isolates and determined the antibiotics susceptibility. This study was done in the Dental Teaching Hospital, Umm Al-Qura University, Makkah, Saudi Arabia. The air samples were

collected from fifteen dental surgery clinics, each clinic by using two different techniques. First technique by using exposure plates which collected from four locations (a) on the tray surface, (b) on the table, (c) front the dental chair, (d) beside the doctor stool. The second technique with using air sampling technique which collected from three locations (i) on the tray surface, (ii) on the table, (iii) front the dental chair.

Maintaining adequate cleanliness in a dental hospital environment is extremely important it affects the health of the patients and medical staff. The viable airborne could come from patients and working staff, which increase the levels of airborne bacterial contamination inside the dental clinic which higher during the treatment than without treatment processes and cause diseases transmission.[18]

From one hundred and twenty air samples collected by exposure plates during the manned conditions, six different bacterial strains were isolated. The most bacterial isolates belonged to Gram positive cocci (47% *Micrococcus* sp and 30% *Staphylococcus* sp), Gram positive rods (22% *Bacillus* sp) and 0.5% Gram negative strains. On the other hand, the fungal isolates were identified as 0.25% *Penicillium* sp and 0.25% *Aspergillus* sp. Also, from ninety air samples collected by air sampler plates during the manned conditions, five different bacterial strains were isolated. The most bacterial isolates belonged to Gram positive cocci (48% *Micrococcus* sp and 29.5% *Staphylococcus* sp) and Gram positive rods (22% *Bacillus* sp). On the other hand, the fungal isolates were identified as 0.25% *Penicillium* sp and 0.25% *Aspergillus* sp.

From one hundred and twenty air samples were collected by exposure plates during the non-manned conditions, six different bacterial strains were isolated.

The most bacterial isolates were belonged to Gram positive cocci (44.5% *Micrococcus* sp and 33% *Staphylococcus* sp) and Gram positive rods (24% *Bacillus* sp). On the other hand, the fungal isolates were identified as 0.25% *Penicillium* sp and 0.25% *Aspergillus* sp. Also, from ninety air samples were collected by air sampler plates during the non-manned conditions, five different bacterial strains was isolated.

The most bacterial isolates were belonged to Gram positive cocci (45% *Micrococcus* sp and 31% *Staphylococcus* sp) and Gram positive rods (23.75% *Bacillus* sp). On the other hand, the fungal isolates were identified as 0.25% *Penicillium* sp. All data was represented in Figure 2.

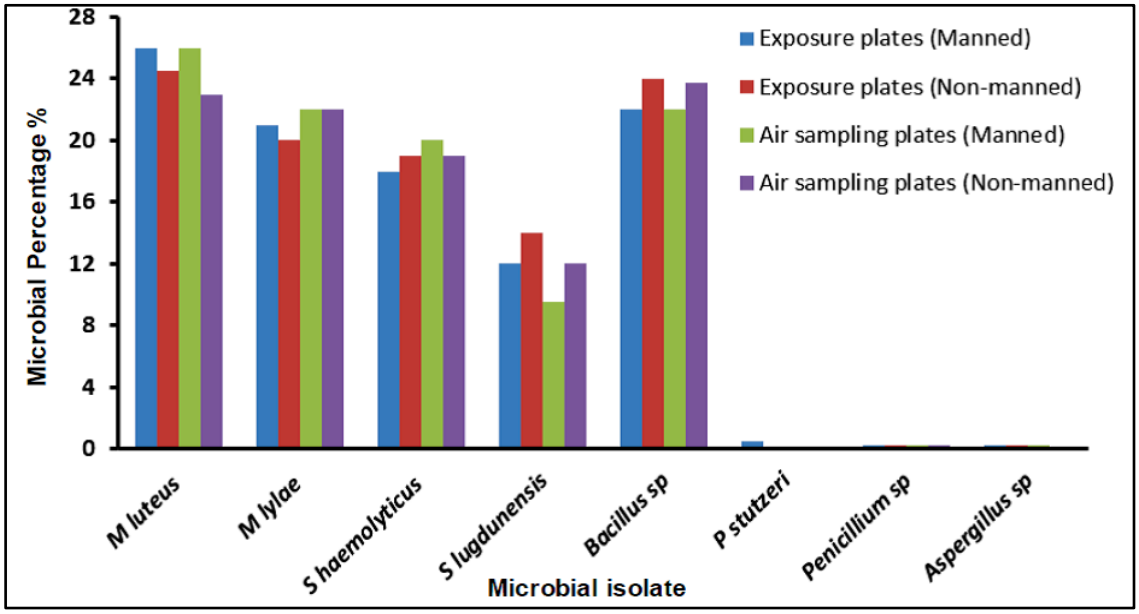

Figure 2: Microbial percentage during the manned and non-manned conditions by using the settle, air sampling plates in DSCs.

Table 1: Microbial air contamination values measured by exposure and air sampling plates during manned and non-manned conditions in DSCs.

No of clinic	Air sampling method	Sampling conditions	Mean	SD	Maximum	Minimum	*P* value
C 35-49	Exposure plates (CFU/h)	Manned	4.9	± 0.1	14	7	0.0003
		Non- manned	0.4	± 0.7	2	0	
C 35-49	Air sampling plates (CFU/m³)	Manned	1	± 1.5	2	0	0.0005
		Non- manned	0.28	± 0.7	1	0	

CFU/m³: colony forming unit per cubic meter, CFU/h: colony forming unit per hour, ± SD: standard deviation.

Pasquarella et al., 2000 were considered the microbial air contamination in the operating room is generally a risk factor for surgical site infections.[13] According to Cellini et al., 2001 the number of airborne bacteria should be observed when compared with non-manned condition in DC.[19] Napoli et al., 2012 and Maher et al., 2017 found a significant correlation between the exposure plates and air sampling methods, while Petti et al., (2003) found a significant correlation between exposure plate and air sampling methods during the high contamination level, but not found during the low contamination level.[20-22] Cellini et al., 2001 determined the microorganisms level in the area of the dental office was present in acceptable value which gave the mean 4-18 CFU/hr.[19] Kedjarune et al., 2000 measured the level of air contamination in Japan by using the air sampler and incubated the plates for 48

hours at 37 °C in aerobically at 5% CO_2 to give 232.49 CFU/m^3.[23] The mean of CFUs were measured by using exposure and air sampling plates during the manned and non-manned condition in different DSCs, recorded in Table 1. The mean values of bacterial and fungal CFUs in the multi DSC was 4.9 CFU/hr during the manned conditions while in non-manned conditions was 0.4 CFU/hr when used exposure plates technique. The mean values bacterial and fungal of CFUs was 1 CFU/m^3 during the manned conditions while in non-manned conditions was 0.28 CFU/m^3. All the results were present within the accepted limits according to the European Union standard for air system. A highly significant correlation (p < 0.001) in manned than non-manned conditions when measured by the exposure plates and air sampling methods were given p value 0.0003 and 0.0005 respectively.

In previous study by Decraene et al., 2008 referred to the importance of preventing cross-infection and hence transmission the antibiotic resistant between DC surfaces and isolated the M. luteus and S. epidermidis at high concentration, while the oral flora such as *Actinomyces* sp, *Streptococcus viridans*, *Haemophilus sp., Neisseria sp.* and *Lactobacillus sp.* at low concentration by using a settle plate in a UK DC.[24] Osorio et al., 1995 isolated the *Streptococcus sp* were accounted between 73-82% in DSCs both non-manned and manned conditions.[25] In a similar study in Japan Noro et al.,1998 found in the airborne DSCs *Micrococcus sp, Streptococcus sp* and *Corynebacterium sp,* reported as 23, 22 and 21% respectively.[26]

Singh et al.,2016 found the CFUs is increased many folds during the operation when compared to pre- and post-operatively, and Yadav et al., 2015 found in previous studies the microorganisms is excess of five times that of outdoor air in dental surgery units.[27,28]

The antimicrobials were tested by VITEK 2 card against *S. lugdunensis* was sensitive against Gentamicin, Levofloxacin, Linezolid, Minocycline, Nitrofurantoin, Oxacillin, Rifampicin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole and Vancomycin, while are intermediate against Fusidic acid and are resistant against Benzylpenicillin, Clindamycin, Erythromycin and Fosfomycin. While *S. haemolyticus*was sensitive against Clindamycin, Erythromycin, Gentamicin, Levofloxacin, Linezolid, Minocycline, Nitrofurantoin, Oxacillin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole and Vancomycin, while was intermediate against Rifampicin, and are resistant against Benzylpenicillin, Fosfomycin and Fusidic acid. All data was collected in Tables 2 and 3.

Table 2: Antimicrobial sensitivity of isolated and identified air bacterial species from DSCs by using exposure plates.

Antimicrobial	Bacterial isolates				
	S. lugdunensis	S. haemolyticus	M. lylae	M. luteus	P. stutzeri
	MIC/In	MIC/In	MIC/In	MIC/In	MIC/In
Negative control	—	—	—	—	—
Amikacin	—	—	—	—	<= 2/S
Ampicillin	—	—	—	—	<= 2/S
Benzylpenicillin	>= 0.5/R	>= 0.5/R	<= 2/S	<= 2/S	—
Cefepime	—	—	—	—	<= 1/S
Cefoxitin	—	—	<= 2/S	<= 2/S	—
Ceftazidime	—	—	—	—	<= 1/S
Ciprofloxacin	—	—	—	—	<= 0.25/S
Clindamycin	>= 8/R	<= 0.25/S	1/S	1/S	—
Colistin	—	—	—	—	<= 0.5/S
Erythromycin	>= 8/R	<= 0.25/S	<= 0.25/S	<= 0.25/S	—
Fosfomycin	>= 128/R	>= 128/R	>= 128/R	>= 128/R	—
Fusidic acid	2/*I	>= 32/R	8/R	>= 32/R	—
Gentamicin	<= 0.5/S	<= 0.5/S	<= 0.5/S	8/S	<= 1/S
Imipenem	—	—	—	—	<= 0.25/S
Inducible Clindamycin resistance	—	—	16/S	8/S	—
Levofloxacin	0.25/S	<= 0.12/S	<= 0.12/S	<= 64/S	<= 0.12/S
Linezolid	1/S	1/S	<= 2/S	<= 2/S	—
Meropenem	—	—	—	—	<= 0.25/S
Minocycline	<= 0.25/S	<= 0.25/S	<= 0.25/S	—	<= 1/S
Moxifloxacin	—	—	—	2/S	—
Nitrofurantoin	<= 16/S	<= 16/S	<= 16/S	<= 16/S	—
Oxacillin	2/S	<= 0.25/S	<= 2/S	<= 2/S	—
Piperacillin/Tazobactam	—	—	—	—	<= 4/S
Rifampicin	<= 0.5/S	<= 0.5/*I	<= 0.5/*I	<= 0.5/*I	—
Teicoplanin	<= 0.5/S	4/S	4/S	<= 1/S	—
Tetracycline	<= 1/S	<= 1/S	<= 0.5/S	<= 0.5/S	—
Tigecycline	<= 0.12/S	<= 0.12/S	<= 32/S	<= 16/S	<= 0.5/S
Tobramycin	<= 1/S	<= 1/S	<= 1/S	<= 4/S	<= 1/S
Trimethoprim/Sulfamethoxazole	<= 0.5/S	<= 10/S	<= 10/S	<= 10/S	<= 20/S
Vancomycin	<= 0.5/S	1/S	1/S	<= 0.25/S	—

MIC are shown in microgram per milliliters, the numeric portion of the MIC is specific for that antibiotics and does not represent a relationship between the potential efficacy of one antibiotics over another, S= sensitive, I= intermediate, R= resistant.

Table 3: Antimicrobial sensitivity of isolated and identified air bacterial species from DSCs by using air sampling plates.

Antimicrobial	Bacterial isolates			
	S. epidermidis	*S. haemolyticus*	*M. lylae*	*M. luteus*
	MIC/In	MIC/In	MIC/In	MIC/In
Negative control	—	—	—	—
Benzylpenicillin	>= 0.25/R	>= 0.5/R	<= 2/S	<= 2/S
Cefoxitin	—	—	<= 2/S	<= 2/S
Clindamycin	<= 0.25/S	<= 0.25/S	1/S	1/S
Erythromycin	>= 8/R	>= 8/R	<= 0.25/S	<= 0.25/S
Fosfomycin	<= 8/S	>= 64/R	>= 128/R	>= 128/R
Fusidic acid	8/*I	<= 0.5/S	8/I	>= 32/R
Gentamicin	<= 0.5/S	<= 0.5/S	<= 0.5/S	8/S
Inducible Clindamycin resistance	—	—	16/S	8/S
Levofloxacin	0.15/S	<= 0.12/S	<= 0.12/S	<= 64/S
Linezolid	1/S	<= 2/S	<= 2/S	<= 2/S
Minocycline	<= 0.25/S	<= 0.25/S	<= 0.25/S	—
Moxifloxacin	—	—	—	2/S
Nitrofurantoin	<= 16/S	<= 32/S	<= 16/S	<= 16/S
Oxacillin	>= 0.25/S	—	<= 2/S	<= 2/S
Rifampicin	<= 0.5/*I	<= 0.5/*I	<= 0.5/*I	<= 0.5/*I
Teicoplanin	4/S	4/S	4/S	<= 1/S
Tetracycline	<= 1/S	<= 16/R	<= 0.5/S	<= 0.5/S
Tigecycline	<= 0.12/S	<= 0.25/S	<= 32/S	<= 16/S
Tobramycin	<= 1/S	<= 1/S	<= 1/S	<= 4/S
Trimethoprim/Sulfamethoxazole	<= 10/S	<= 10/S	<= 10/S	<= 10/S
Vancomycin	2/S	<= 0.5/S	1/S	<= 0.25/S

MIC is shown in microgram per milliliters, the numeric portion of the MIC is specific for that antibiotics and does not represent a relationship between the potential efficacy of one antibiotic over another, S= sensitive, I= intermediate, R= resistant.

Genet et al., 2011 isolate *S. aureus* and *S. pyogenes* from air sampling from surgery rooms and found all *S aureus* isolates showed 100 and 82.8% resistance to methicillin and ampicillin respectively.[29] Also, Solomon et al., 2017 were isolated *S aureus*, *Enterococci* sp, *Enterococcus faecalis*, *Enterococcus faecium*, *Acinetobacter* sp, *E coli* and *P aeruginosa* from airborne hospital environment and found *Acinetobacter* species showed a high rate of resistance against Trimethoprim-sulfamethoxazole, Gentamicin and Ciprofloxacin, while *S aureus* isolates were Methicillin resistant, and Enterococci isolates were Vancomycin resistant.[30]

M. luteus and *M. lylae* are sensitive against Cefoxitin, Erythromycin, Gentamicin, Levofloxacin, Linezolid, Moxifloxacin, Nitrofurantoin, Oxacillin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamet and Vancomycin, while are intermediate against Rifampicin and resistant against Benzylpenicillin, Fosfomycin and Fusidic acid. All data was collected in Tables 2 and 3. *P. stutzeri* was sensitive against Amikacin, Ampicillin, Cefepime, Ceftazidime, Ciprofloxacin, Colistin, Gentamicin, Imipenem, Levofloxacin, Meropenem, Minocycline, Piperacillin/tazobactam, Tigecycline and Trimethoprim/sulfamethoxazole. While, *S. epidermidis* was sensitive against Clindamycin, Fosfomycin, Gentamicin, Levofloxacin, Linezolid, Minocycline, Nitrofurantoin Oxacillin, Teicoplanin, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole and Vancomycin, while are intermediate against Fusidic acid and Rifampicin and are resistant against Benzylpenicillin and Erythromycin. Also, *S. haemolyticus* was sensitive against Clindamycin, Fusidic acid Gentamicin, Linezolid, Levofloxacin, Minocycline, Nitrofurantoin, Teicoplanin, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole and Vancomycin, while are intermediate against Rifampicin, and are resistant against Benzylpenicillin, Erythromycin, and Tetracycline. All data was collected in Tables 2 and 3.

The Antifungal susceptibility was tested by agar disk diffusion method according to El banna et al., 2014, against, *Aspergillus* sp and *Penicillium* sp were sensitive to Batrafen, Canasten, Flucoral, Fungican and Mycosat antifungal. All data was presented in Table 4.[17]

Table 4: Antifungal susceptibility of *Aspergillus* sp and *Penicillium* sp isolates from DSC air samples.

Fungal isolates	*Aspergillus sp*	*Penicillium sp*
	Inhibition zone diameters (mm) / Interpretation	
Batrafen	41/ S	44 / S
Canasten	45 / S	40/ S
Flucoral	42/ S	42/ S
Fungican	18/ S	44/ S
Mycosat	46/ S	48/ S

0: Resistant; < 20: Low sensitive; 20 – < 40: Moderately sensitive; ≥ 40:Highly sensitive.

CONCLUSION

The microbiological air quality in the dental hospital is a very important parameter to control healthcare associated infections, and regular microbial air monitoring and surveillance is a useful tool to assess the air quality and to identify critical situations which require corrective intervention. The microbial air quality and quantity can be monitored by exposure and air sampling plates methods according to the European Union standard for the air system. So, the air quality of DSC environment, in restricted settings deserves attention, and requires long-term surveillance to protect both patients and healthcare workers.

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