SEYBOLD Report
ISSN: 1533 - 9211
DOI: 10.5281/zenodo.7221768

ANTIBACTERIAL EFFICACY OF FISH LIVER OIL ON THE TYPE OF BACTERIA AND ITS COUNT IN DENTAL AEROSOLS

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Introduction

Dentists, dental hygienists and oral health care workers practice in a highly sensitive environment with marked contamination where they are exposed to a variety of bacteria, viruses, fungi and protozoan from many sources [1]. Transmission of infection during any dental treatment can occur through different routes such as direct contact with blood, saliva or tissue debris, indirect contact with contaminated instruments or surfaces that are improperly sterilized or contact with infective agents present in either the droplets or aerosol particles from saliva and respiratory fluids.[2] During dental treatments, saliva may become aerosolized and microorganisms from the mouth will contribute to the spread of infection.[3] Dental handpieces, ultrasonic scalers, air polishers and air abrasion units produce the foremost visible aerosols. Each of these instruments produces an aerosol from the operative site by the action of the rotary instrument, ultrasonic vibrations or the combined action of water sprays and compressed air. Aerosol transmission is one of the possible modes of contamination when there is exposure to high concentrations of aerosols in a relatively closed environment. Routine dental procedures generate aerosols that can pose potential risks to the dental care personnel and also to the patients. Dental teams should be well aware of the risks and maintain a healthy environment for preventing such infections. Therefore, understanding aerosol transmission and its implications in dentistry are essential. In addition to standard precautions, some special precautions should also be implemented. [4] Bacterial species like *Pseudomonas aeruginosa, Pseudomonas cepacia, Legionella pneumophila* and *Mycobacterium chelonae* are identified in biofilms in dental clinics.[5] The concentration of total bacterial aerosols is said to clinical working hours in dental surgeries.[6] The emergence of the novel coronavirus disease 2019 (COVID-19) caused by the highly infectious SARS- CoV-2 virus at the end of 2019 led the World Health Organisation (WHO) to declare a global pandemic on 11 March 2020.[7]

Multi-drug resistance is common and increasing among Gram-Negative Bacilli (GNB) and several strains are being identified that exhibit resistance to commonly used antibiotics, including antipseudomonal penicillins, cephalosporins, aminoglycosides, tetracyclines,

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fluoroquinolones, trimethoprim-sulfamethoxazole, and carbapenems (McGawan et al., 2006).[8] The impact of antibiotic resistance on the outcome of infections due to GNB remains highly controversial. Therapeutic options for multi-drug resistant GNB strains are limited, for this reason, there is a continuous need for alternative new chemical entities and their activities may be identified through a variety of approaches (Combes et al., 2007).[9]

Unsaturated fish liver oils have high nutritional values and are used widely in the food and pharmaceutical industries. They possess high levels of polyunsaturated fatty acids and vitamin A and D. [10,11]. Although fish liver oil has numerous beneficial properties, its antimicrobial activities have been least studied. Gorgiev in 1960 found that oxidized fish oils have antimicrobial properties. [12] There are fewer or almost no studies published on the antimicrobial activity of fish liver oil. Hence the present study is aimed to determine the antibacterial effect of the fish liver oil on dental aerosol bacteria around the dental unit during the dental procedure.

Materials and Methods

The study was conducted in two school dental clinics (clinic I - Department of Endodontics and clinic II – Department of Periodontics) and data was collected. Both the dental clinics were well equipped with a good air-conditioned indoor environment. A total of 15 uniformly divided cabins were present that had 15 dental chairs each. The clinics received 80 patients with fallout samples (N 80).

Clinics I and II had a total of 30 chairs and all of them were used for the study placing 5 agar plates at each chair (150 plates) at a distance of 1 – 1.5 m from the patient's mouth. Other than these, two other separate plates were kept as negative controls in the Central Research Laboratory department (CRL) where there were no fallout samples seen. Sheep blood agar plates were used for sample collection during the procedures. The placement of plates in each chair is mentioned as follows,

1. Fish Liver oil 100%
2. Fish Liver oil 50%
3. Fish Liver oil 25%
4. Positive control
5. Positive control

Fish Liver oils were applied to the plates by the spread plate technique. In Clinic-I and II, the plates were kept open for 30 minutes. After sample collection, the plates were transported to the CRL department and were incubated at room temperature 37°C for 48 hours. The colonies were seen macroscopically and microscopically. Using the Bergers manual, the type of colonies and bacterial species were identified. It was followed by performing biochemical tests to determine the pathogenicity of the species present in the agar plates.

Fish Liver Oil

The Fish Liver oil in its purest form was shipped through Amazon. For all the concentrations,

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a standard mixture quantity of 150µl was used to perform the spread plate technique and their various concentrations were diluted using Dimethyl Sulfoxide (DMSO) as given below.

Fish Liver oils and DMSO ratio (150 µl)

100% - 150 µl: 0 (Full strength)
50% - 75µl: 75µl (oil: DMSO)
25% - 50µl: 100µl (oil: DMSO)

Results

Clinic-I

Type of Bacteria	Bacterial Name	Percentage
Gram-Positive Rod	Bacillus	6.64%
Gram-Negative Rod	Escherichia coli	41.32%
Gram-Positive Cocci	Staphylococcus sp.	33.32%
Gram-Negative Rod	Klebsiella sp.	18.8%

Clinic-II

Type of Bacteria	Bacterial Name	Percentage
Gram-Positive Rod	Bacillus	7.56%
Gram-Negative Rod	Escherichia coli	56.25%
Gram-Positive Cocci	Staphylococcus sp.	16.54%
Gram-Negative Rod	Klebsiella sp.	19.65%

A vast amount of bacterial contamination was seen in all the sample plates that were placed both at the operatory site with and without fish liver oil. Figures 1 & 2 shows the results

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of the bacterial fallout collection during dental procedures. The mean density of aerobic oral bacteria was 990 CFU/m^2/h from each plate in Clinic - I and 1330 CFU/ m^2/h from every plate in Clinic - II. There was no significant difference in the number of bacterial colonies present in any of the samples.

Finally, in Clinic-I we observed the total contamination of Gram-negative rods which consisted of *Escherichia coli* species (41.32%) and *Klebsiella* species (18.8%). Apart from that we also found Gram-positive cocci, namely *staphylococcus* species 33.32%, and Gram-positive rods, namely *Bacillus* species (6.64%). Whereas in clinic-II the Gram-negative rods were found in abundant and most prominent ones were *Escherichia coli* species (56.25%) followed by Gram-positive cocci, namely *staphylococcus* species (16.54%) and the other two were Gram-negative rods, namely *Klebsiella* species (19.65%) and Gram-positive rods, namely *Bacillus* species (07.56%). In both the clinics, Escherichia *coli* was found at a higher percentage as compared to other bacteria. Negative Control plates which were placed in the CRL department did not show any bacterial colony formation.

Discussion

Aerosols containing particles more than 50 μm in diameter are referred to as spatter, whereas particles measuring less than 50 μm are called droplet nuclei. Because gravitational pull causes spatter aerosols to settle very quickly on surfaces which are less likely to hold microorganisms that induce infection. Droplet nuclei, however, remain suspended within the air for several hours and may infect persons by direct inhalation and penetration deep into the lungs. Larger 10 -15 μm droplet nuclei particles are closely related to upper respiratory infections, while smaller 0.5 - 5 μm droplet nuclei can accumulate in the lower respiratory tract and may cause viral respiratory infections.[13,14]

The aerosol production in during dental procedures using ultrasonic and sonic scalers, Headpieces and air polishers are well documented in many dental studies. These instruments require a water spray as a coolant to inhibit heat production. A water spray is also used to lavage the working area to increase the operator's vision. The water spray from hand piece of the instrument can mix with the patient's saliva or blood to make a potentially infective agent. The pressure of high speed airotors and scalars, both being used in combination with a water could generate numerous airborne particles derived from blood, saliva, tooth debris, dental plaque, calculus, and restorative materials.[15] Hence in the present study was carried out in department of endodontics and periodontics where such high speed instruments are in use.

The results of the current study revealed the presence of different forms of bacteria like Gram-positive cocci, namely *Staphylococcus*, Gram-negative bacilli, namely *Klebsiella,* and *Escherichia* species in both the dental clinics. This finding was in concurrence with the study done by (Rautemaa et al., 2006) in dental aerosol contamination within a distance of 1- 1.5m.[16] There were no significant differences in the bacterial count present in plates with and without fish liver oil. This result was not concurrent with the study carried out by Savoiskii in 1938 where he concluded that fish liver oil possessed antibacterial activity[17]. It is unlikely that fish liver oil did not possess any antibacterial activity in any of the concentrations. The absence of

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any significant differences in outcome between the two groups may be because the oil might have acted as a medium for the growth of bacteria. If the fish liver oil was indeed efficacious we can conclude that it would be used as an antimicrobial agent against dental aerosols. Blinov 1995[18] also concluded that an antimicrobial preparation of fish liver oil provided excellent results which were not in concurrence with the current study. As there are no recent literature pieces of evidence regarding the antimicrobial activity of fish oil, furthermore studies are essential to conclude.

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