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Detection & Quantification of Microorganisms in Dental Unit Waterlines

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Abstract: *The aim of the study was to assess microbial quality of water in dental unit waterlines in a dental school at Mangalore. Methodology: Water samples were taken from tap water for control, air/water syringe and the high speed hand piece of 20 dental units. The samples were transferred to the laboratory and streaked on the culture media namely Mac Conkey agar, Sabourauds dextrose media, Buffered charcoal yeast extract agar base & Lactobacillus agar. The microorganisms were identified by gram staining followed by the biochemical methods and the number of colony forming units (CFU) was determined.*

Results: *The results were tabulated using One-way ANOVA test. The maximum microorganisms found in the samples obtained from the handpiece belonged to the species in the following order Legionella (142.8 CFU), Lactobacillus spp (120.3 CFU) , Acinetobacter spp (93.25 CFU),Micrococcus spp (82.55 CFU), Staphylococcus spp (73.4 CFU), Pseudomonas aeruginosa (67.85 CFU), Klebsiella pneumonia (64.1 CFU), Streptococcus spp (56.8 CFU), Burkholderia cepacia(0 CFU). The samples obtained from the air/water syringe showed Legionella (116.45 CFU) ,Lactobacillus spp(115.2 CFU) , Acinetobacter spp(95.75 CFU) , Micrococcus spp (80.35 CFU),Staphylococcus spp (70.85 CFU),Klebsiella pneumonia(62.6 CFU), Pseudomonas aeruginosa (52.9 CFU) ,Streptococcus spp (52.35 CFU), Burkholderia cepacia (0 CFU).The samples obtained from the control group showed Legionella (160.55 CFU), Lactobacillus spp (140.9 CFU), Acinetobacter spp (130.95 CFU),Micrococcus spp (93.35 CFU),Staphylococcus spp (84.15 CFU), Pseudomonas aeruginosa (94.7 CFU), Klebsiella pneumonia (75.4 CFU),Streptococcus spp (59.65 CFU), Burholderia cepacia (0 CFU)*

Conclusion: *The Dental unit water lines showed microbial count well within the permissible limits specified by the American Dental Association (ADA).*

Keywords*: Air/water syringe ,Biofilm, Dental Unit Waterlines, Handpiece, Microorganisms.*

I. Introduction

Aquatic biofilms are well organized communities of microorganisms, which are widespread in nature. They constitute a major problem in many environmental, industrial & medical settings.1In dentistry, the surface of the dental unit water lines(DUWL) provide an ideal environment in the development of a microbial biofilm. The sources for microorganisms include municipal water piped into the dental units and suck back of patient's saliva into the waterline due to lack of preventive valves .2 The necessity for preventive microbiological maintenance of dental units' water line is given because of the ambient water temperature in the water lines , the synthetic material of water lines supporting biofilm formation together with curves and kicking leading to stagnation ,because of dead spaces and because of water stagnation during non-operational times . The combination of the above conditions is an ideal prerequisite for biofilm formation in case of bacterial contamination of the indwelling water. Biofilms, harboring non-pathogenic and pathogenic micro-organisms, are a source for continuous contamination of the cooling water in dental unit water lines3. Microbiologically contaminated water maybe a risk factor for the dental team & the patient since they are both exposed to water & aerosols generated from dental units. 4,5

All dental procedures involving the use of handpiece cause aerosols and splatter which are commonly contaminated with microorganisms including potential pathogens6.Numerous studies have emphasized the need to reduce microbial contamination in dental unit waterlines.

The structure of the dentals units favor the biofilm formation and microbial contamination of the dental unit waterlines. According to current knowledge it is not the mere presence of microorganisms that is important, but the number of microorganisms and the presence of potential pathogens. The American Dental Association(ADA) recommendations for Dental Unit Waterline quality (DUWL) is**<200 CFU/ml.** If the CFU/ml is above the prescribed range the water would not be considered fit for human consumption7.

Hence this study was conducted to detect and quantify microorganisms from dental unit waterlines of various specialty departments of a dental school in Mangalore.

II. Materials & Methods

Water samples were taken from air/water syringe and high speed handpieces of 20 dental units at the dental school. 100 ml of water samples were collected aseptically in sterile containers at the beginning of the day after a 2 minute purge. 20 water samples were taken from the tap water as control group. Samples were then transferred to the laboratory and immediately processed in the following manner. The culture media was prepared in petri plates. Each sample was centrifuged and 0.1 ml of the sediment was taken. Every sample was then streaked on the following culture media MacConkey agar, Sabourauds dextrose media. Buffered charcoal yeast extract agar base,Lactobacillus agar using the loop technique.After incubation the identification was concentrated mainly on the following microorganisms by Gram staining followed by biochemical methods of identification and the number of colony forming units (CFU) was determined for the same ,Acinetobacter spp,Legionella,Klebsiella pneumonia,Lactobacillus spp,Micrococcus spp,Pseudomonas aeruginosa,Burkholderia cepacia,Streptococcus spp,Staphylococcus spp.

1.1 Gram staining

Gram staining is a differential staining technique that differentiates bacteria into two groups: gram positive and gram negative. The procedure was based on the ability of microorganisms to retain color of the stains used during the gram stain reaction. Gram-negative bacteria are decolorized by the alcohol, losing the color of the primary stain, purple. Gram-positive bacteria are not decolorized by alcohol and will remain as purple. After decolorization step, a counter stain is used to impart a pink color to the decolorized gram-negative organisms.

Bacterial smear was prepared and heat fixed. The slide was filled with crystal violet for 1 minute. Excess stain was poured off and gently washed in with running water. Gram's iodine was put and left for 1 minute and washed with water. The smear was washed with 95% alcohol for 30 seconds and again washed with water. It was counter stained with 0.25% safranin for 30 seconds and washed with water. The smear was then drained, blotted and examined under oil immersion microscope. If the bacteria shows pink color then it is considered as Gram negative. If the cells stained purple then it is concluded Gram positive bacteria. Colonies are subcultured onto non selective nutrient media and used for further biochemical identification.

1.2 Biochemical identification

Acinetobacter is identified based on the biochemical reactions; they are catalase positive, oxidase negative, nonmotile and utilize many substrates. The both species appear as coccobacilli on Gram strain. They produced a pale yellow to white-greyish pigment on the solid medium. The colonies were not pigmented.

Legionella is identified using the buffered charcoal yeast extract agar. After 10 days of incubation the growth of Legionella is confirmed.

Klebsiella are Gram negative lactose fermenting bacteria.It metabolizes glucose with rhe production of gas.It appears as mucoid colonies in MacConkey agar. They show positive reaction for indole, methyl red (MR) and negative result for Vogues-Proskauer test and citrate test.

Micrococci are Gram-positive spherical cells ranging from about 0.5 to 3 micrometers in diameter and typically appear in tetrads. They are catalase positive, oxidase positive, indole negative and citrate negative. Micrococcus produce yellow or pink colonies when grown on Mannitol salt agar.

P. aeruginosa is often preliminarily identified by its pearlescent appearance and grape-like or tortilla-like odor in vitro. P aeruginosa secretes a variety of pigments, pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown).

Burkholderia cepacia are Gram negative , lactose non fermenters, catalase producing organisms. Morphology, and hemolysis were observed and oxidase activity was tested. A heavy emulsion in saline was made, and a drop was placed in Hugh and Leifson oxidation-fermentation sugar. Bacteria were incubated in the following sugars for up to 7 days at 35°C glucose, maltose, xylose, and sucrose. A modified lysine and ornithine decarboxylase medium (containing 5g/l glucose, 5g/l KH_2PO_4, 5g/l lysine monochloride and 0.1g/l bromocresolpurple; pH= 4.6± 0.2) and negative controls were also heavily inoculated and incubated at 35°C for two days. Organisms were also tested for the presence of betagalactosidase(O-nitrophenol-β-galactopyranoside ONPG). In addition, growth on trypticase soy agar at 35° and 42°C was observed for appearance and pigmentation.

Streptococci are non-motile, gram positive spherical bacteria (cocci). They often occur as chains or pairs and are facultative or strict anaerobes. Streptococci give a negative catalase test, while Staphylococci are catalase-positive. Beta hemolysis is a true hemolysis of erythrocytes by the enzyme hemolysin. Clear zones will appear around the colonies on the blood agar plate. Coagulase test is performed Staphylococcus aureus shows positive reactions.The results obtained were then statistically analyzed using ONE WAY ANOVA TEST.

III. Results And Discussion

The quantitative bacterial analysis of water samples collected from the dental units for handpiece and air/water syringe indicated that the dental units under study delivered water that could meet the accepted standard of American Dental Association(ADA) dental unit water quality(<200 CFU/ml).Table 1 shows the results in CFU/ml for both air/water syringe and handpiece.).The results show that samples taken from the hand piece showed the maximum CFU's of microorganisms in the decreasing order Legionella (142.8 CFU) followed by Lactobacillus spp(120.3 CFU) ,Acinetobacter spp(93.25 CFU),Micrococcus spp(82.55 CFU),Staphylococcus spp(73.4 CFU),Pseudomonas aeruginosa(67.85 CFU),Klebsiella pneumonia(64.1 CFU) and Streptococcus spp(56.8 CFU).However the CFU in Burkholderia cepacia showed zero for the samples from handpiece.(Table 1,Fig.1)The samples taken from the air/water syringe showed results almost similar as that of the handpiece Legionella(116.45 CFU) followed by Lactobacillus spp(115.2 CFU) , Acinetobacter spp(95.75 CFU) ,Micrococcus spp(80.35 CFU),Staphylococcus spp(70.85 CFU),,Klebsiella pneumonia(62.6 CFU), Pseudomonas aeruginosa(52.9 CFU) and Streptococcus spp(52.35 CFU).Similarily the CFU in Burkholderia cepacia showed zero for the samples obtained from the air/water syringe.(Table 1,Fig.1).The samples obtained from the control group showed Legionella(160.55 CFU),Lactobacillus spp(140.9 CFU),Acinetobacter spp(130.95 CFU),Micrococcus spp(93.35 CFU),Staphylococcus spp(84.15 CFU),Pseudomonas aeruginosa(94.7 CFU),Klebsiella pneumonia(75.4 CFU) and Streptococcus spp(59.65 CFU)Burholderia cepacia was zero similar to the handpiece and air/water syringe values There was no significant statistical difference in the number of CFU of the water samples from the handpiece and air/water syringe. However there was an increase in the number of CFU in the control group when compared to the handpiece and air/water syringe.The decresed CFU in the handpiece and air/water syringe is due the the filtering system in the dental uni

IV. Tables

Table 1.Mean colony forming units(CFUs') of microorganisms obtained from handpiece and air/water syringe.

Microrganisms(CFU)	Acinetobacter spp	Legionella	K.pneumonia	Lactobacillus. spp	Micrococcus spp	Pseudomonas aeruginosa	B.cepacia	Streptococcus spp	Staphylococcus spp
C	130.95	160.55	75.4	140.9	93.35	94.7	0	59.65	84.15
HP	93.25	142.8	64.1	120.3	82.55	67.85	0	56.8	70.85
A/W	95.75	116.45	62.6	115.2	80.35	52.9	0	52.35	93.29

CFU- Colony forming units
C- Control group
HP - Hand piece group
A/W- Air water syringe group

V. Discussion

Aerosols and droplets produced by dental instruments connected to dental unit waterlines (DUWLs) during dental care may contain microorganisms that can be opportunistic pathogens for patients and dentists. Microbial proliferation inside DUWLs is inevitable and is principally associated with biofilm formation. It represents a low but current risk of infection . This becomes quite significant when immunocompromised patients (the elderly, smokers, HIV+ or cancer patients, people with diabetes, alcoholism, etc.) are treated 10. The dental unit water can also be heavily contaminated with opportunistic pathogens that can pose a major risk for the dental team and the patients11.The microorganisms included in the study are usually found in the public water systems and hospital/clinical environment. These microorganisms cause sepsis, pneumonia, periodontitis, oropharyngeal infection and other noscomial diseases.12

In the present study ,142.8 CFU of Legionella were found in the samples obtained from the handpiece and 116.45 CFU in the samples obtained from the air/water syringe which is in agreement with a study conducted by Atlas et al where Legionella spp. was detected in 68% of the samples and L.pneumophila was detected in 8%. 13 A study conducted by Challacombe et al also showed the presence of Legionella pnemophila in Dental unit waterlines. 15 Legionella is a gram negative organism which is usually found in public water distribution systems. It causes Hospital Acquired Legionella Pneumonia which has a fatality rate of 28%. Infection is initiated by inhalation of the aerosols containing high levels of Legionella.14, 15

In our study,67.85 CFU of Pseudomonas aeruginosa were found in the samples obtained from the handpiece and 52.9 CFU in the samples obtained from the air/water syringe. This is in concurrence with a study conducted by Al Hiyasat et al which showed that Pseudomonas aeruginosa thrive commonly in Dental unit

waterlines. A study by Barbeau et al reported that Pseudomonas aeruginosa was isolated from 24% of the tested waterlines. 5In another study conducted by Stampi et al ,there was a notable growth of Pseudomonas aeruginosa was observed in the dental unit waters.12Pseudomonas aeruginosa is an opportunistic ,nosocomial pathogen which affects immunocompromised patients more commonly.16Pseudomonas aeruginosa is present in the oral cavity and can be aspirated to the dental unit waterlines.

The microbial samples taken from the dental handpiece varied from 0 to 142.8 CFU/100 ml whereas the samples taken from the air/water syringe varied from 0 to 116.4 CFU/100ml.In a study conducted by Mahnaz Nikaeen et al the number of CFU in the water samples from handpiece and air/water syringe varied from 0 to 540 CFU/ml and 0 to 770 CFU/100ml16 respectively,while the study conducted by Szymanska et al had reported a variation between 0 and 375 CFU/ml in water flowing from high speed handpieces.8

The variation in the results could be attributed to several reasons such as source of water supply, infection control measures at different centres and methodology of microbial assessment.

VI. Conclusion

The nature of Dental unit waterlines is such that they will develop a biofilm and water flowing through it will contribute to the microbial load. Both dentists and patients are at risk to infections from exposure to aerosols as the bacterial load in dental unit waterlines contributes to potential pathogens.9

The quantitative bacterial analysis of water samples collected from the dental units for handpiece and air/water syringe indicated that the dental units under study delivered water that could meet the accepted standard of American Dental Association(ADA) dental unit water quality(<200 CFU/ml).Since the phenomenon of dental unit waterlines contamination is more clearly defined now better progress can be made by the dental manufacturers and the scientific community in approaches to the prevention and control.

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Abbreviations
DUWL : Dental unit waterlines
CFU : Colony forming units
ADA : American Dental Association