International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 5 Sep-Oct 2023, pp: 435-449 www.ijprajournal.com ISSN: 2249-7781

Evaluation of Microbial Contamination in Dental Unit Water Reservoirs - An in vitro study

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Submitted: 15-09-2023 Accepted: 25-09-2023

ABSTRACT: Dental units use water for various dental procedures. The dental unit water system (Dental unit waterlines and dental unit water reservoir bottle) are sites for microbial contamination and the development of biofilms due to the presence of narrow water line tubings and long periods of water stagnation in the water reservoir. The presence of microorganisms poses a significant health risk as these may come into contact with the patient and the clinician during a dental procedure. The aim of this study is to assess and compare the microbial levels in the dental unit water reservoir from 4 different clinical departments. Samples were collected from 20 in-use dental units selected randomly from 4 clinical departments, based on inclusion and exclusion criteria. Reservoir bottles of dental units were randomly selected for sampling. Samples were collected in the form of swabs. The swabs were collected in a screw cap bottle with peptone water and incubated for 4 hours. Then, subculture was done from swabs onto Nutrient agar & Macconkey agar and kept for 24 hours incubation to detect the presence of aerobic organisms. Samples were also collected and put in Robertson Cooked Meat Medium, then subculture was done onto blood agar to detect the presence of anaerobic organisms.Data were recorded, tabulated, and entered in Microsoft Excel (v. 2013) for infographic representation.

Results showed out of 20 water reservoir bottles, 11 bottles were found with microbial growth. The major microbial growth was caused by non-pathogenic microorganisms, whereas the pathogenic microorganisms were present within permissible limits.The organisms which were observed in samples are Enterobacter species, Escherichia coli, Micrococcus species, Streptococcus species, Pseudomonas species, Aerobic spore bearers, Proteus Vulgaris, Fungi & Algae.

Conclusions: The present study, clearly shows that the dental unit water reservoirs also invariably possess a lot of aerobic organisms and insignificant levels of anaerobic organisms. Hence, it is mandatory to use appropriate disinfectants for cleaning and weeky maintaining the dental unit waterlines and water reservoirs.

KEYWORDS: Dental unit water contamination, Microbial contamination, Dental unit waterlines disinfection.

I. INTRODUCTION

Disinfection is a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects.It is mandatory to follow appropriate infection control protocols by dental professionals as inappropriate disinfection and sterilization practices can cause a breach between the host and the infective agent[1]

Pathogens can be transmitted from human skin, inanimate surfaces, medical devices, dust, or a moist environment.[2] The risk of infection in dental care settings can be attributed to the unique nature of dental procedures, which include aerosol generation, handling of sharp instruments, and proximity of the provider to the patient's oropharyngeal region.[11]

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Water should also be considered an important source of infection due to the numerous occasions of exposure to the complex hospital water systems as well as water-containing machinery used in dental clinics.[3] The water supplied through dental unit waterlines (DUWL) has various applications during dental procedures. The air-water syringes, ultrasonic scalers, high-speed air turbine handpieces, and water reservoirs are connected to dental units by a network of small-bore plastic tubes through which water and air travel to activate or cool the instruments.[4] This narrow-bore tubing presents a very large ratio of surface area to volume (6:1), which encourages biofilm formation.[5] Contamination is often observed in the complex DUWL with high densities of microorganisms, such as bacteria, fungi, viruses, and protozoa.[5,6,7]

The Centers for Disease Control and Prevention (CDC) guidelines recommend that the bacterial count in DUWLs water should not exceed 500 colony-forming units (CFUs)/ml.[8] Opportunistic pathogens such as Legionella pneumophila, Pseudomonas aeruginosa, Mycobacterium, Staphylococcus aureus, and amoebae have previously been revealed in water samples from DUWL[5,9]. In addition, other genera such as Propionibacterium and Stenotrophomonas were also recovered in dental unit waters.[10]

These pathogens pose a health risk, especially to immunocompromised patients. Patients and dental healthcare personnel are exposed to pathogens in dental unit water during every procedure. Several reports have reported diseases associated with DUWL, especially pneumonia caused by Legionella pneumophila[11,12] Fan et al recently reported Facial cutaneous sinus tract associated with Mycobacterium fortuitum, M. abscessus, and M. peregrinum in the DUWL.[13]

Literature reveals microbial contamination in the waterlines of dental units. However, there is a paucity of evidence of microbial growth and level of contamination in the water reservoirs of the dental unit. Therefore, the current study was undertaken to assess and compare the microbial levels in the dental unit water reservoir from 4 different clinical departments.

II. MATERIALS AND METHODS :

This experimental study was conducted to assess and compare the microbial levels in the dental unit water reservoir from 4 different clinical departments.

Area of study: This pilot study was conducted in our institution. In collaboration with the Clinical departments and Department of Microbiology.

Samples were collected from 4 Clinical departments of the same institution. 20 Dental units from 4 different clinical departments which met the inclusion and exclusion criteria were taken for this study. Dental units in working conditions that are routinely used for aerosol procedures were included and dental units in poor/not working conditions not used for aerosol procedures were excluded from the study. 20 dental units with independent reservoirs were selected from 4 clinical departments and were labeled as group A, B, C and D.

Study Groups :

Group A: Department 1- R 1 to R5 (n=5)
Group B: Department 2- R 6 to R10 (n=5)
Group C: Department 3- R 11 to R15 (n=5)
Group D: Department 4- R 16 to R20 (n=5)

5 reservoir bottles from each of the 4 clinical departments were collected. The independent reservoir bottles were labeled from R1 to R20. Samples were collected in the form of swabs from the reservoir bottles. The swab was rubbed onto the inner surface of the reservoir bottle in a swirling motion for 20 seconds for sample collection. The collected swabs were placed in a screw cap bottle with peptone water and incubated for 4 hours. The screw cap tubes were UV sterilized prior to sample collection. For the identification of microorganism microbial culture, gram staining, biochemical tests were done. Also the colony morphology and motility were observed to identify the microorganism. The biochemical tests which were done included Catalase test, IMVIC test, Carbohydrate fermentation test, Oxidase test and Urease test.

Microbial Culture: Subculture was done from swabs onto Nutrient agar &Macconkey agar and kept for 24 hours incubation to detect the presence of Aerobic organisms. Samples collected were kept in Robertson Cooked Meat Medium, then subculture was done onto blood agar to detect the presence of anaerobic organisms.

Gram staining: A smear was prepared of suspension on the clean, grease-free slide with a loopful of the sample, which was air dried and heat fixed. Crystal Violet was poured and kept for about 30 seconds to 1 minute and rinsed with water. The

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gram's iodine flooded for 1 minute and washed with water. Then, washed with 95% alcohol for about 10-20 seconds and rinsed with water. Safranin was added for about 1 minute and washed with water. Air-dried, Blot dried, and Observed under a Microscope.

Catalase test: Using a sterile glass rod, several colonies were taken of the 18 to 24 hours test organism and immersed in the hydrogen peroxide solution, and observed for immediate bubbling.

Indole test: A sterile test tube containing 4 ml of tryptophan broth was taken and inoculated aseptically by taking the growth from 18 to 24 hrs culture. The test tube was incubated at 37°C for 24-28 hours. 0.5 ml of Kovac's reagent was added to the broth culture and observed for the presence or absence of a ring.

Methyl red test: Using organisms taken from an 18-24 hour pure culture, the medium was lightly inoculated. Then incubated aerobically at 37°C for 24 hours. Following 24 hours of incubation, aliquot 1ml of the broth to a clean test tube. Reincubating the remaining broth for an additional 24 hours. Adding 2 to 3 drops of methyl red indicator to aliquot and observed for red color immediately.

Voges-Proskauer test: Using organisms taken from an 18-24 hour pure culture,the medium is lightly inoculated. Incubated aerobically at 37°C for 24 hours. Following 24 hours of incubation, aliquot 2 ml of the broth to a clean test tube. Re-incubate the remaining broth for an additional 24 hours. 6 drops of 5% alpha-naphthol added, and mixed well to aerate. Then 2 drops of 40% potassium hydroxide were added, and mixed well to aerate. Observed for a pink-red color at the surface within 30 min. The tube is vigorously shaken during the 30-min period.

Citrate Utilization test: Streaking the slant back and forth with a light inoculum picked from the center of a well-isolated colony. Incubated aerobically at 35°C to 37°C for up to 4-7 days. Observed fora color change along the slant.

Carbohydrate Fermentation Test (Sugar Fermentation Test): The Purple Broth (with carbohydrate of choice) was inoculated with isolated colonies from an 18-24 hour pure culture of the organism. A control tube of Purple Broth Base was also inoculated in parallel with the carbohydrate based media. Incubating the inoculated media aerobically at 35°C-37ºC for 3-5 days. Observed daily for development of a yellow color in the medium.

Oxidase test: A fresh culture (18 to 24 hours) of bacteria in 4.5 ml of nutrient broth (or standard media that does not contain a high concentration of sugar) is grown. 0.2 ml of 1% α-naphthol, then 0.3 ml of 1% p-aminodimethylaniline oxalate (Gaby and Hadley reagents) is added. Shaken vigorously to ensure mixing and thorough oxygenation of the culture. Observed for color changes.

Urease test: Inoculating slant with 1 to 2 drops from an overnight brain-heart infusion broth culture. Leaving the cap on loosely and incubating the tube at 35°C-37°C in ambient air for 48 hours to 7 days. Observed for the development of a pink color for as long as 7 days.

Identification of fungal colonies: Identification of fungi is performed by observing various aspects of colony morphology, characteristic microscopic structures, rate of growth, media which supports the organism's growth, and source of specimen.

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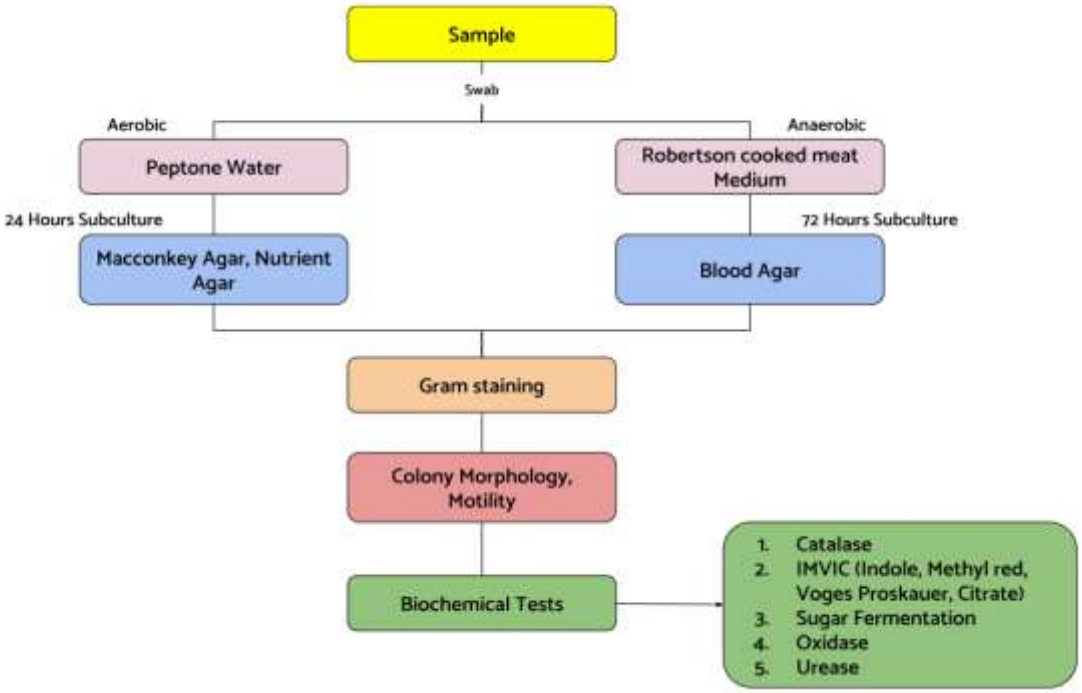
FLOWCHART:



Flowchart 1. Methodology

MICROBIAL COLONY COUNTING:

Manual colony counting followed by automatic colony counting was done. Manual counting of colonies was done by counting the colonies under light illumination. First step is placing the culture plate onto a light box. Dividing the plate into grids for ease of counting. Using a sharp-tipped marker each colony is marked as we count it. To increase accuracy in counting, the plate is rotated 90° and counted again; & a second person to confirm each plate's tally. Also an application named Promega's Colony Counter was used to counter check the colony count. The application allows you to photograph agar plates and automatically counts the colonies, thereby providing a rough estimate.

CALCULATION:

CFU = (nc*DF)/vc

Colony Forming Unit per ml = No. of Colonies*Dilution Factor/Volume of Culture Plate.

No. of Colonies is a group of bacteria, fungi, and other microorganisms grown on a solid agar medium, Dilution Factor is the factor by which the stock solution is diluted and volume of Culture Plate is the amount of a substance occupying a particular volume of culture plate.

Figure 1: Reservoir bottles collected for study

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Figure 2: UV sterilization of armamentarium

Figure 3: Peptone water

Figure 4: swirling of swab for sample collection

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Figure 5: Storage of samples in peptone water

Figure 6: Inoculation of swab in robertson cooked meat medium

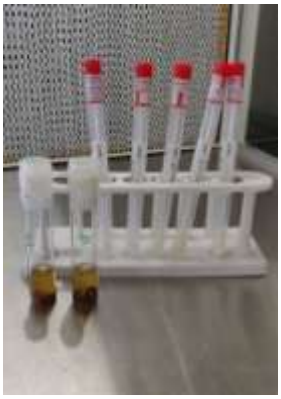

Figure 7: Sample subjected to anaerobic culture

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Figure 8: Incubation of samples

Figure 9: Subculture for aerobes

Figure 10: Subculture for anaerobes

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III. RESULTS

Out of 20 water reservoir bottles, 11 bottles were found with microbial growth. The major microbial growth was caused by non-pathogenic microorganisms, whereas the pathogenic microorganisms were present within permissible limits.The organisms which were observed in samples are Enterobacter species, Escherichia coli, Micrococcus species, Streptococcus species, Pseudomonas species, Aerobic spore bearers, Proteus Vulgaris, Fungi & Algae. The overall results of the microbiological investigation are depicted in a pie chart 1.

Microorganisms in Water reservoirs of Clinical Department 1 :

Water Reservoirs collected from clinical department 1 were numbered 1-5. Out of the 5 collected reservoirs, 4 reservoirs had microbial growth and one did not have any growth. Majorly the routine procedures performed are aerosol-generating procedures. The major microbial growth observed was Micrococcusspecies followed by the aerobic spore bearers, Algae, E.coli, and Fungal colonies as shown in Graph 1.

Microorganisms in Water reservoirs of Clinical Department 2 :

Water Reservoirs collected from clinical department 2 were numbered 6-10. The routinely performed procedures are less aerosol generating when compared to department 1. 3 reservoirs had microbial growth and 2 did not show any growth out of 5 reservoirs The major microbial growth observed was by Enterococcusspecies followed by aerobic spore bearers, algae, and Proteus Vulgaris as shown in Graph 2.

Microorganisms in Water reservoirs of Clinical Department 3 :

Water Reservoirs collected from clinical department 3 were numbered 11-15. The routinely performed procedures majorly include aerosol generating procedure when compared to department 2. The major microbial growth observed was by Aerobic spore bearers and Micrococcus species. Mild growth of E.coli was present. 3 reservoirs had microbial growth out of 5. Absence of fungal and algal in all 5 reservoirs as shown in Graph 3.

Microorganisms in Water reservoirs of Clinical Department 4 :

Water Reservoirs collected from clinical department 4 were numbered 16-20. The routinely performed aerosol generating procedures are very minimal when compared to other clinical departments. Only 2 reservoirs had microbial growth and 3 did not have any growth out of the 5 reservoirs collected. Micrococcus species followed by Enterococcus species caused the major microbial growth, and minimal levels of algae, streptococcus, and pseudomonas species were evident as shown in Graph 4.

Figure 11: Culture Plates

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Figure 12: Micrococci

Figure 13: E.Coli and fungal colonies

Figure 14: Enterobacter spp, Proteus Vulgaris

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Figure 15: Enterobacter spp

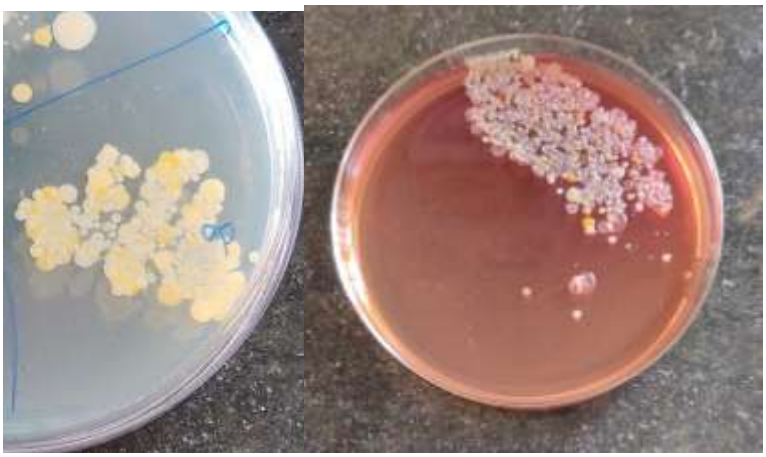

Figure 16: Aerobic spore bearers

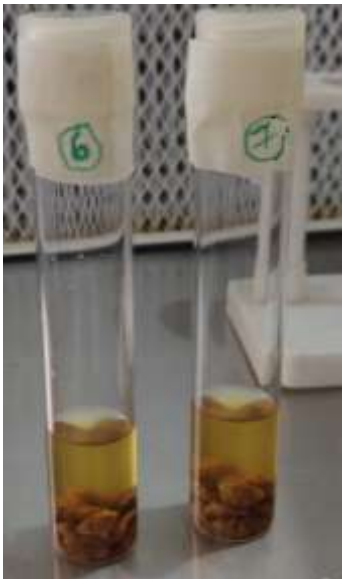

Figure 17: Anaerobes in Robertson cooked meat medium

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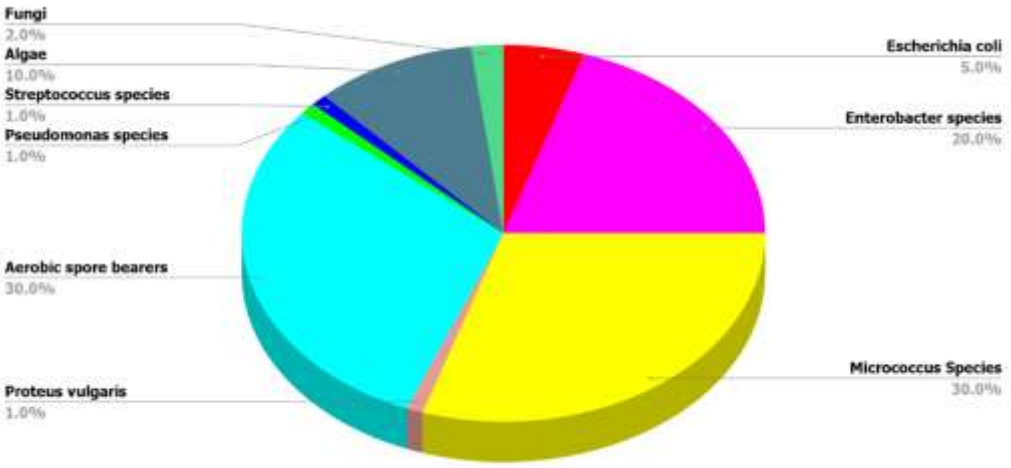
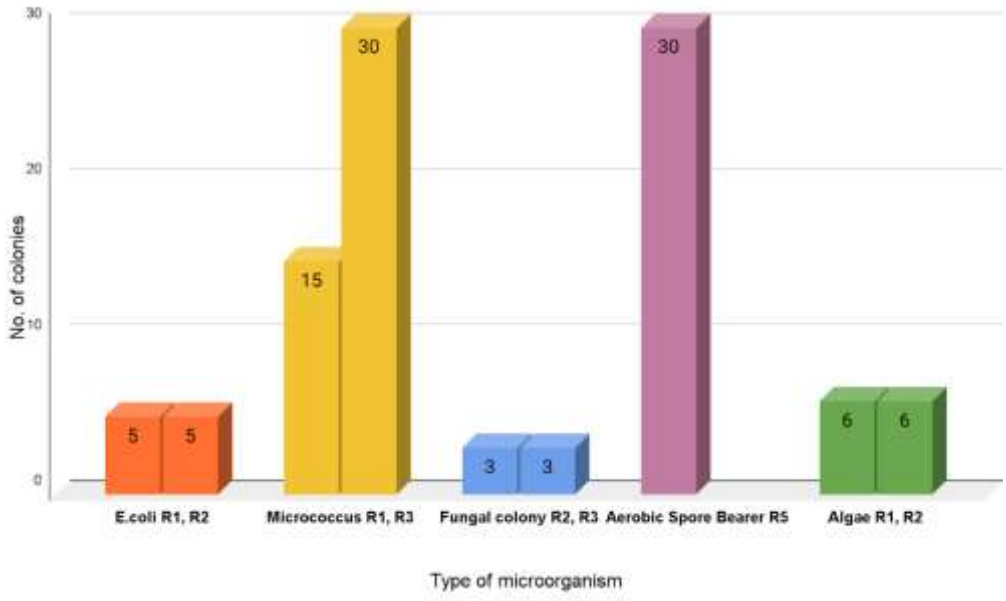
PIE CHART AND BAR GRAPHS:



Pie chart 1. Microorganisms found in Water reservoirs (R1-R20)



Graph 1. Microorganisms in Water reservoirs of Department 1 (R1-R5)

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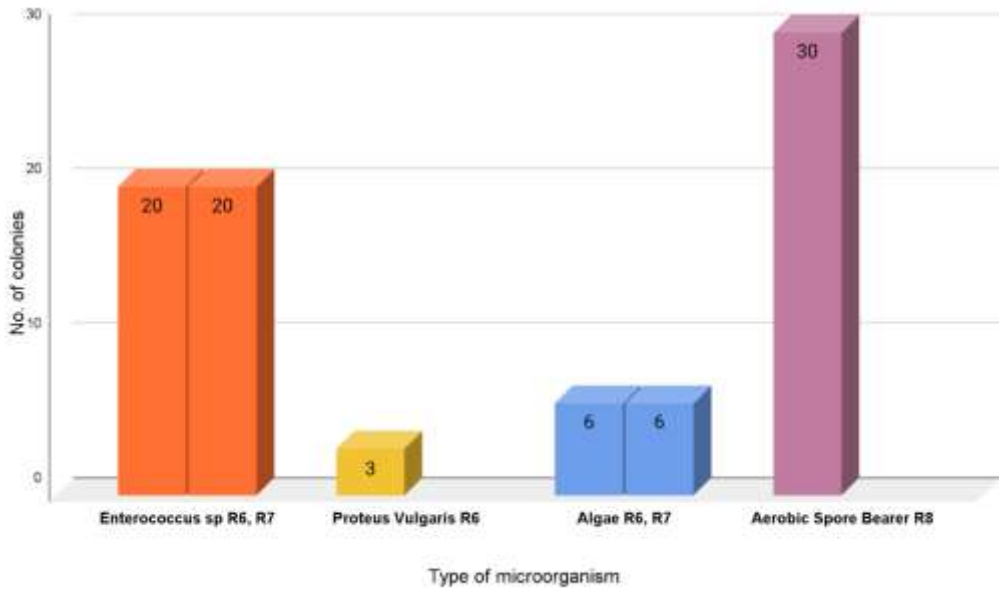
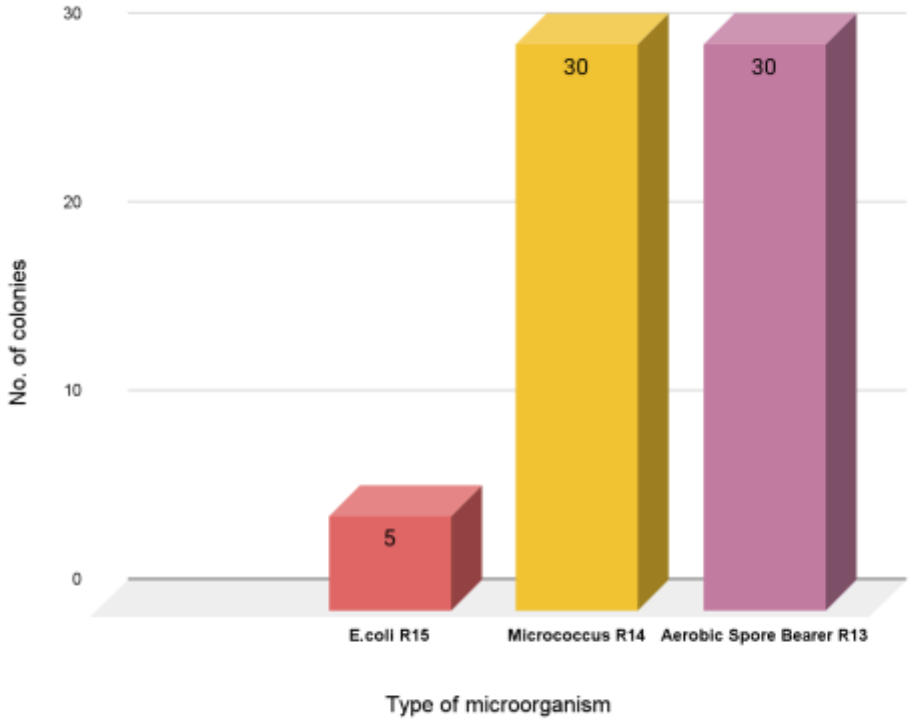


Graph 2. Microorganisms in Water reservoirs of Department 2 (R6-R10)



Graph 3. Microorganisms in Water reservoirs of Department 3 (R11-R15)

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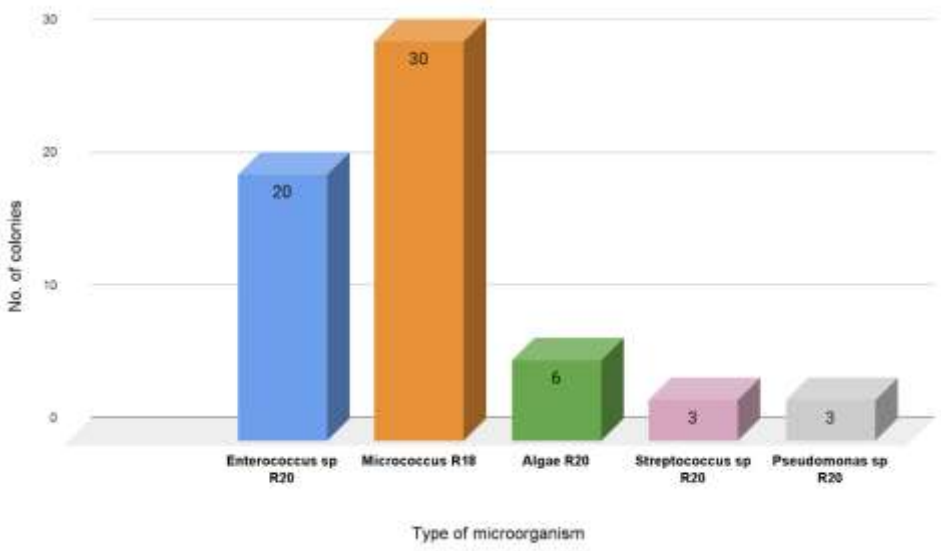

Graph 4. Microorganisms in Water reservoirs of Department 4 (R16-R20)

IV. DISCUSSION

A dental unit waterline (DUWL) is a complex system that delivers water to water reservoir bottles, spittoon glasses for patients, handpieces for high-speed drills, ultrasonic scalers, and air and water syringes. Water is used to cool dental instruments and also to irrigate tooth surfaces during dental procedures, as the heat that is generated during usage can be harmful to teeth. Water from DUWLs can also be used for oral rinsing to wash out the dental chair unit spittoon, or cuspidor, after oral rinsing (water supplied via the bowl- rinse outlet)[3]

Dental unit water reservoirs are independent or removable water reservoir systems now available as an integral part or as an accessory for most dental-units. By isolating the dental-unit from the municipal water system the quality of water introduced in the system can be controlled (and in addition the municipal water system is protected from contamination). This separate reservoir allows chemical agents to eliminate or inactivate biofilm organisms to be readily introduced into the DUWL.[14]

DUWL contamination is often caused by the water supplied to the dental unit and the oral cavities of patients by aspiration of biological fluid during therapy.

Biofilm in DUWLs is caused by different factors, such as water stagnation in reservoirs when patients are not treated, Laminar flow of water that passes through a DUWL is maximal at the center of the lumen and less at the periphery, which favours the deposition and adhesion of microorganisms to the inner surface of the tube and, thus, promotes biofilm formation. Other factors anti-retraction valves failure, the presence of water heaters (maintaining temperatures over 20 °C), and variations in the type of water supply (tap water, distilled water, or sterile water).[3,15]

First report revealed the presence of microbial contamination in water coming from dental units. The opportunistic pathogens identified in dental treatment water include Pseudomonas, Moraxella, Klebsiella, Legionella and Mycobacterium.[16,17,18]. From the DUWL. Gram-negative species such as Enterobacter spp., P. vulgaris, and P. aeruginosa and gram positive species such as Streptococcus spp. were identified and revealed the presence of bacteria morphologically resembling C. tetani.[19]

DUWL contamination: opportunistic pathogens such as Streptococcus spp., Enterococci spp., Pseudomonas aeruginosa, Legionella spp., and other gram-negative rods isolated from these lines can cause pneumonia, other respiratory infections, or wound infections in immunocompromised people.[16,18,20,21,22] Several studies have revealed the presence of microbial contamination in dental unit waterlines and potential health hazards to patients and healthcare

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personnel. Whereas there is a paucity of evidence related to contamination in dental unit water reservoirs.

The present study was done to observe the microbial growth and type of organism present in dental unit water reservoirs. The type of organism helps us to select the appropriate disinfectant. Water reservoirs were collected from various departments and organism present were detected

The organisms which were revealed in the present study are Aerobic spore bearers, micrococcus species, Enterobacter species, Algae, Escherichia coli, Fungi, Streptococcus species, Pseudomonas species, Proteus vulgaris. Based on the amount of water utilized from reservoirs for dental treatment, the microbial growth varied in each department. Dental units with stagnant water in reservoirs favoured microbial growth when compared to frequently used dental units.

Limitations of the study include sample size, standardization of units, and disinfection protocol variation in departments.

V. CONCLUSION

The dental unit water reservoirs possess aerobic organisms and insignificant levels of anaerobic organisms irrespective of stringent disinfection procedures. The present study clearly shows that not only dental unit waterlines but also the dental unit water reservoirs are contaminated and require periodic maintenance to provide water of good quality for dental procedure. CDC & ADA has given few recommendations for maintenance of waterline. Which includes, usage of water that meets EPA regulatory standards for drinking water (i.e., ≤ 500 CFU/mL of heterotrophic water bacteria) for routine dental treatment output water, discussion with the dental unit manufacturer for appropriate methods and equipment to maintain the quality of dental water and to follow monitoring of water quality provided by the manufacturer of the unit or waterline treatment product.

Dental clinics should undergo a sterilization process which should also include fumigation followed by screening for the bacterial spores in waterlines and reservoirs of dental units. Lack of spores is the indication of thorough sterilization of the dental clinics and hence the safety of patients. Several disinfection methods available are chemical methods using different disinfectants such as peracetic acid, hydrogen peroxide, silver salts, chloramine, glutaraldehyde, chlorhexidine, chlorine dioxide, EDTA, and sodium hypochlorite[23], ozonation of water, anti-retraction devices in dental turbines, and auto-flushing dental units and Ultraviolet disinfection method.

Establishing an Infection control team for periodical maintenance by checking for microorganisms and using appropriate disinfectants that meet EPA standards for disinfecting the dental unit waterlines and water reservoirs. Along with periodical replacement of the unsterile reservoir bottles with sterilized ones.

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