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RESEARCH ARTICLE

ISOLATION OF AEROBIC MICRO-ORGANISM FROM DENTAL UNIT WATERLINES ATTACHED TO A ULTRASONIC SCALER DEVICE

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ABSTRACT

Background: Bio films form speedily on dental unit waterlines. The specific design of dental units favors bacteria to colonize surfaces and to form biofilm in water supply tubes, together with dental unit water line (DUWL). In recent years, microbial contamination of DUWLs has become a prominent infection control issue even receiving some alarmist coverage in the media. More than 25 different species of bacteria have been isolated from DUWLs. It may also harbour opportunistic pathogens that are responsible for respiratory disease – namely P. aeruginosa, Legionella spp and non–tuberculous Mycobacterium spp.

Aim: To determine the predominant bacteria isolated from DUWL biofilm attached to ultrasonic scaler device.

Method: A total 10 sample were collected from department of period ontology, TKDC & RC, Kolhapur. Each tube was aseptically cut into 2 cms length. The biofilm was removed by scraping the wall with a scalpel and the scraping were suspended in peptone water. Each suspension were spread on agar plate and incubated for 48 hrs. To obtain pure culture, morphologically different colony forming unit (CFU) were selected for subculture.

Finding: The following aerobic organisms were found in samples of DUWL.

Proteus species, Bacillus species, Staphylococcus albus, Staphylococcus citreus, Staphylococcus aureus.

Conclusion: From the DUWL, the microorganism are transmitted with aerosol and splatter, generated by working unit handpieces. Numerous studies highlight the need for effective mechanisms to diminish the microbial contamination in DUWL and emphasize the threat for cross-infection in general practice, especially in view of the ever-increasing number of persons with immunocompromised state who present at outpatient dental clinics.

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INTRODUCTION

Biofilms are known to be implicated as major cause of infection and in the pathogenesis of several diseases (Costerton, 1987). It has been entranched that water used in dental treatment has high microbial counts, typically ranging from 10^4 to $>10^6$ CFU/ml (Costerton, 1987). Past studies addressing dental unit water supply (DUWS) contamination have confirmed that the high bacteria count is due to the exfoliation of biofilm bacteria from the lumen surface of dental waterline tubing into treatment water (Tall, 1995). Many of the microorganisms in dental unit water lines are not pathogenic in healthy individuals but, may be of great importance in patients with systemic disease (Szymanska, 2003).

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The relatively large surface area/volume ratio associated with the dental unit waterline (DUWL) tubing, periods of stagnation (when the dental unit is not in use) of water in the lines, and luminary state conditions with low shear forces near the lumen wall of the waterlines provide the opportunity for the bacterial biofilms to develope. The principle content of the biofilm, the exopolysaccharide, dispenses added protection to the biofilm organisms by limiting the dispersion of antimicrobial agents and they acts as a nutrient source for the bacterial community (Costerton, 1999). The existence of bacterial biofilms also increases resistance to flow in the DUWL and can act as a reservoir for potential pathogens. Contamination of DUWL can be of huge importance since the patients and dental personnel are in regular contact with the water and aerosols that are produced in the environment (Szyman´ ska, 2004). The numerous microorganisms seperated from DUWL are potential opportunistic pathogens such as Streptococci spp, Legionella,

Staphylococci spp, Pseudomonas aeruginosa, Enterococci spp, and other gram negative rods (Ajami, 2009). These micro-organisms can cause respiratory infections, pneumonia, or wound infections in immunocompromised people. Although the outcome of various other epidemiologic studies showed that contamination of DUWL can be dangerous in patients which are prone to immune-deficiency, it can also be true for pregnant women, elderly, graft recipients or even smokers. Dental recruits have been shown to have changed nasal flora, with the colonization of Pseudomonas spp. consistent with those found in their dental units (Morrison, 1986). In 1996, the American Dental Association (ADA) set a endeavor for dental water to contain not more than 200 colony-forming units per milliliter (cfu/ml) of heterotrophic unfiltered output (ADA Council on Scientifi c Affairs,1999).

In 2003, the Centre for Disease Control and Prevention (CDC) suggested ≤ 500 cfu/ml for non-surgical dental procedures (CDC,2003). In the European Union (EU), there are no specific standards for dental unit waterlines (DUWL), but it was recommended in the guidelines that water should be delivered at <100 cfu/ml at 22°C and <20 cfu/ml at 37°C (Council Directive 98/83/EC, 1998). Concentration and composition of microflora in water and in the biofilm in dental units is the basis for assessment of contamination of the DUWL by microbial species. Detection of microorganisms, mainly of bacterial species, is based on usual laboratory criteria (haemolytic zones, colony morphology, production of catalase and coagulase), with the use of biochemical tests and modern methods of molecular biology. The present study was conducted with the aim to determine the predominant aerobic bacteria isolated from DUWL biofilm attached to ultrasonic scaler device in Tatyasaheb Kore Dental College & Reaserch Centre, Kolhapur.

MATERIALS AND METHODS

The current study was conducted in the department of Periodontology, TKDC and RC, Kolhapur. Prior consent before the commencement of study was taken from concerned authorities. Samples were taken from all the ten chairs in the department. The tubing samples were obtained from a total of 10 dental units (Figure-1) that were in use 5 to 7 years. The portion of the DUWL closest to the patient, which is approximately 3 ft long, was selected. After removal from the dental unit, the tubing was transferred to the laboratory in less than 5 min for analysis.

Fig. 1. Dental units

Fig. 2. Each tube aseptically cut into 2-cm lengths

Each tube was aseptically cut into 2-cm lengths (Figure 2). Four sections were arbitrarily selected and cut longitudinally to expose the inner surface. The biofilm was removed by scraping the wall with a scalpel, and the scrapings were suspended in Peptone water & incubated at 37^0c for 48 hours (Figure,3). Then they were subcultured on nutrient agar by conventional method and then plates incubated in incubator at 37°c for 48hrs (Figure 4). Then next day plates were observed and results were documentated (Figure 5 & 6).

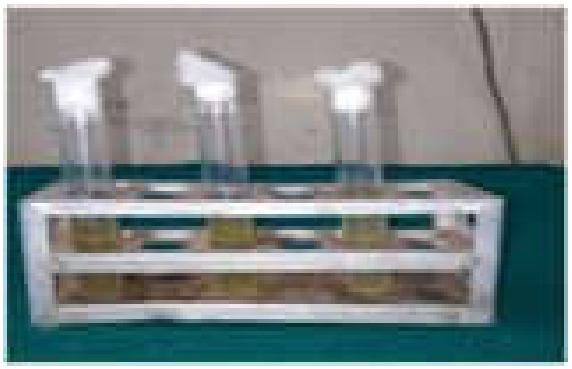

Fig. 3. Scrapings suspended in Peptone water

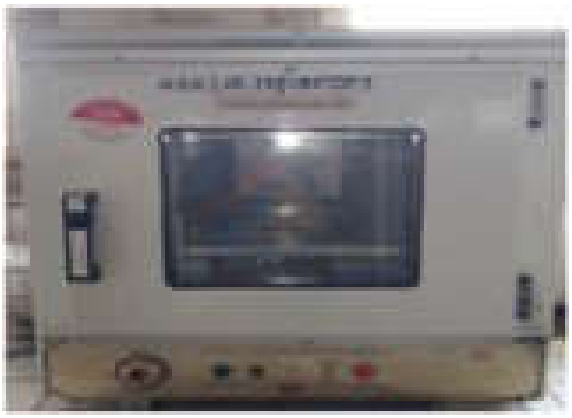

Fig. 4. Incubation at 37°c for 48hrs

Observation

The following aerobic organisms were found in samples of DUWL.

1. Proteus species (1 sample).
2. Bacillus species (1 sample).
3. Staphylococcus albus (5 sample).
4. Staphylococcus citreus (1 sample).
5. Staphylococcus aureus (2 sample). (Figure-5 & 6)

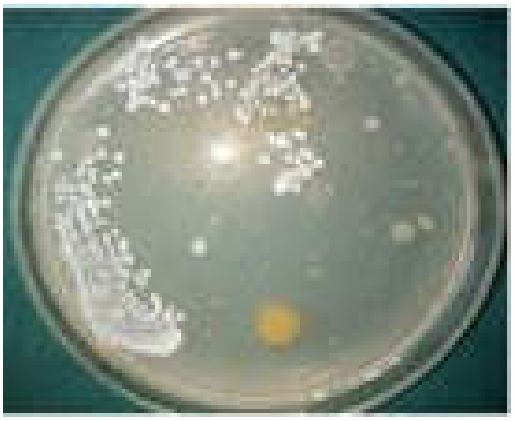

Fig.5. Colony of Staphylococcus epidermidis on nutrient agar

Confirmation of above bacteria were done by routine conventional method. E.g. Catalase test, Coagulase test, Penicillinase test, Sugar fermentation test in case of

Staphylococcus and for Proteus species various biochemical test like Sugar fermentation test.

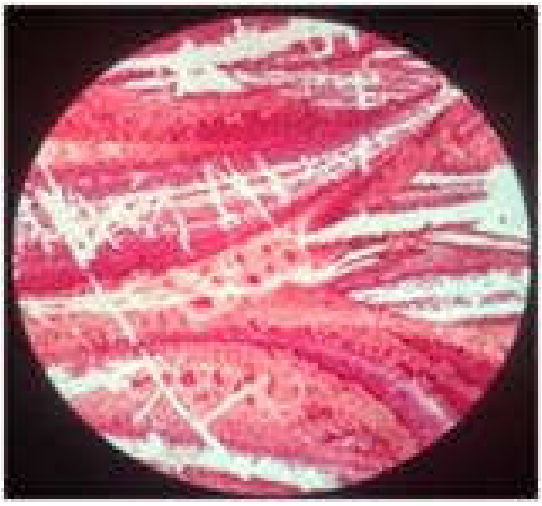

Fig. 6. Gram stain shows Gram positive cocci in cluster

DISCUSSION

The present study was conducted in Tatyasaheb Kore Dental College and Research Centre, Kolhapur (Maharashtra) to assess the quantitative contamination of dental unit water lines**.** In all the samples, only species detected were in the dental unit water lines was coagulase negative staphylococci. The presence of staphylococci coagulase negative in the dental unit waterlines has also been reported by (Venkatash, 2006), (Lachachi Meriem, 2014) and by (Messano Giuseppe A,2013) in the dental environment. The source of microorganisms in DUWL may be suck back of patient's saliva into the line due to the lack of preventive valves (Singh, 2003). In the present study, sterile distilled water was used in the department; hence the possibility of detection of microorganisms was excluded. The next possibility was suck back of patients saliva into the line due to lack preventive valves (Singh, 2003), but the chairs installed in the department were fitted with anti-retraction valves.

So, the only hypothesis that can be drawn from here is that the anti retraction valves are not very effective. As coagulase-negative staphylococci are mostly associated with opportunistic human and they colonize mucosal surfaces beside with Staphylococcus aureus which may cause a risk for infection in immune compromised states **(**Shailee Fotedar, 2015). So, advanced research are required to recognize the actual source of coagulase negative staphylococci (Staph. epidermidis) and to take suitable measures to prevent it in order to avoid any kind of cross infections. The staphylococci present in all the samples and for that needs a suitable disinfection procedures in order to avoid any cross infections **(**Shailee Fotedar, 2015). Centers of Disease Control recommended about flushing the dental unit water lines alone is not consistent procedure for improving water quality used in dental treatment. A rational procedure for disinfecting is required so that water used for dental patient treatment satisfies accepted safe public health standards. The drawback of this study is that the anaerobic culture methods. For a species like legionella and free living amoeba need a special method and which is not studied. So, additional research with above mentioned methods and to find out the actual source of staphylococcus coagulase negative are recommended.

Conclusion

The definite constitution of dental units favors the presence of biofilm and microbial contamination of the DUWL water. The capability of bacteria to colonize surfaces and to form biofilm in water supply tubes, including DUWL, is a common phenomenon, which has been well accepted, just as with difficulties in biofilm removal and prevention of its regrowth. From the DUWL, the microorganism are transmitted with aerosol and splatter, generated by working unit handpieces. The most of the microorganism which are isolated from the DUWL are of low pathogene city. Yet, the public health importance of many of the microorganisms found in DUWL is indefinite. According to current research, presence of bacteria, their number, the presence of potential pathogens, and patient oral cavity micro flora is important in DUWL contamination monitoring. Numerous studies highlight the need for effective mechanisms to diminish the microbial contamination in DUWL and emphasize the threat for cross-infection in general practice, especially in view of the ever-increasing number of persons with immunocompromised state who present at outpatient dental clinics.

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