Research Journal of Microbiology 2 (12): 983-987, 2007
ISSN 1816-4935

Prevalence of *Pseudomonas aeruginosa* in Dental Unit Water-Lines

D.H. Tambekar, P.B. Gulhane, K.S. Goyal and S.R. Gulhane
Department of Microbiology, S.G.B. Amravati University, Amravati-444602, India

Abstract: The quality of dental unit water is of considerable importance since patients and dental staff are regularly exposed to water and aerosols generated from the dental unit waterlines (DUWLs). *Pseudomonas aeruginosa* contamination in dental unit waterlines was reported by its origin from incoming local water supplies of the dental clinics. A total of 82 dental unit water samples from 34 dental clinics of Amravati city were collected, out of which 59 water samples were contaminated by *P. aeruginosa*. The ultrasonic scaler showed maximum 81% contamination of *P. aeruginosa* then other two hand-pieces, 3 in 1 syringe 68% and air rotor 69%. The dental clinics using distilled water as a source of water for treatment showed 72% *P. aeruginosa* contamination whereas Undergraduate (UG) dentist's clinics showed 75% and Postgraduates (PG) dentist's clinics showed 68% *P. aeruginosa* in DUWLs water.

Key words: Dental unit waterlines, biofilm, infection, microbial contamination, *Pseudomonas aeruginosa*

INTRODUCTION

The quality of dental unit water is of considerable important since patients, staff are regularly exposed to water and aerosols generated from the unit. The contamination of Dental Unit Waterlines (DUWLs) is of great concern to the dental profession, since the water in these lines has the capacity for rapid development of biofilms combined with the generation of potentially contaminated aerosols (Forde *et al*., 2005; Walker *et al*., 2004). The water obtained from dental units via 3-in-1 syringes, air rotors and low-speed hand pieces may be heavily contaminated with microbial pathogens and thus may be a potential source of infection for both practice staff and patients. Water entering DUWLs is usually free from pathogens; but after shedding of bacteria from the biofilm, it becomes contaminated over the acceptable level (Rautemaa *et al*., 2006).

A wide range of organisms have been isolated from DUWL which include fungi, free living amoebae, protozoa, nematodes, *Pseudomonas* species, *Klebsiella* species and *Flavobacterium* species (Al-Hiyasat *et al*., 2007; Monteiro *et al*., 2003). In addition there are opportunistic and true human pathogens such as *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Mycobacterium* species and *Staphylococcus* species (Mavridou *et al*., 2006; Pankhurst *et al*., 2003) *P. aeruginosa* derived from DUWL has definitively been reported to give rise to infections in immunocompromised patients (Martin, 1987). Certainly DUWL seem to be a potential source of sub-clinical infection in dental health-careworkers and medically compromised dental patients due to formation and subsequent sloughing off of microbial biofilms from the surface of tubing within DUWLs (Walker *et al*., 2000).

The biofilms play an important role in the microbial contamination of water systems and *P. aeruginosa* from biofilm that colonize DUWLs contaminate the water that is used in dental treatment, which violates basic infection control principles. Thus, the objective of this study was to determine the occurrence of *P. aeruginosa* contamination in dental unit waterlines (reservoir, triple-syringe and ultra sonic scaler) in dental clinics and to aware dental staffs and patients from the infection caused by the bacterial pathogen in dental water, which is used during dental surgical procedures.

Corresponding Author: D.H. Tambekar, Department of Microbiology, S.G.B. Amravati University, Amravati-444602, India

Table 1: Antibiotics used in the study

Antibiotics	Concentration (mcg)
Amikacin (Ak)	30
Cefaclor (Cj)	30
Ciprofloxacin (Cf)	5
Gatifloxacin (Gf)	30
Erythromycin (E)	15
Nitrofurantoin (Nf)	300
Novobiocin (Nv)	30
Piperacillin (Pc)	100

MATERIALS AND METHODS

A total of 82 dental unit water samples from 34 dental clinics of Amravati city were collected in the period of 6 months from June to December 2006. The water collected from 3 in 1 syringe 28, Air rotor 32 and Ultrasonic scaler 22 in sterilized plastic water sample collection bottle by flushing the water for 15 sec before collection. One milliliter dental water sample of each clinic from different sources was inoculated in 9 mL MacConkey broth and incubated at 37°C for 24 h. A loopful inoculum from MacConkey broth was subcultured on MacConkey agar and cetrimide agar plates (Hi-Media Laboratories, Mumbai). These plates were incubated at 37°C for 24-48 h. Plates were observed for growth and a Gram smear was performed from different types of colonies. Gram reaction, colony morphology, pigment formation, florescence, catalase, coagulase, urease and oxidase tests were performed and allocated to appropriate genera to the isolates. The cultural characteristics including lactose fermentation by enterobacteriaceae on MacConkey agar, pyocynin formation of *Pseudomonas* sp. on cetrimide agar were noted. Further identification to species level was carried out on the basis of various specialized tests (Collee *et al*., 1996).

All the confirmed *P. aeruginosa* strains were subsequently tested for antibiotic sensitivity patterns by Bauer *et al*. (1966) disk diffusion method using antibiotics discs obtained from Hi-Media Laboratories Pvt. Ltd Mumbai. The isolates were considered antibiotics resistant if the zone of inhibition was 10 mm or less. The antibiotic susceptibility pattern of *P. aeruginosa* strains was determined on the day of their isolation by the Bauer *et al*. (1966) disk diffusion method on Muller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials (Table 1). Finally, the data were recorded and analyzed at the completion of the study as per recommendations of the Anonymous (2000). *P. aeruginosa* MTCC 424 was used as reference strain for the standardization of antibiotic susceptibility testing. The Multiple Antibiotic Resistant (MAR) index was determined by procedure described by Krumperman (1983).

RESULTS

A total of 82 DUWLs water samples from 34 dental clinics were collected and screened for isolation and identification *P. aeruginosa*. Out of these, 72 clinics used distilled water and 10 clinics overhead tank water by 19 postgraduate and 63 undergraduates qualified dentist's clinics. On analysis, 59 DUWLs water samples showed presence of *P. aeruginosa* from 52 distilled water (40 U.G. qualified and 12 P.G. qualified dentist clinics and 7 overhead tank water supply (all U.G. qualified dentist clinics). Out of 59 *P. aeruginosa* isolates, 19 from 3 ways syringe, 22 from Air rotor and 18 from Ultrasonic scaler. Out of total 82 DUWLs water samples, 15 53% water samples from different hand-pieces of 8 U.G. qualified dentist clinics whereas 7 57% belonging to 4 P.G. qualified dentist clinics were found to be free from *P. aeruginosa*. The 59 isolates of *P. aeruginosa* were isolated from dental clinics where frequency of washing of water container was daily 17, twice a day 8, once in two days 22, once in three days 2, once in four days 3 and weekly 7 (Table 2).

Table 2: Presence of *P. aeruginosa* in various DUWL water samples

				Hand piece type			
P. aeruginosa	Type of water	Dentist's qualification	Frequency of washing	3 ways syringe	Air rotor	Ultrasonic scaler	Total
Absent (23) (28%)	Distilled water (20)	UG (13)	Daily	3	3	2	8
			Once in 2 days	1	2	1	4
			Weekly	0	1	0	1
			Total	4	6	3	13
		PG (7)	Daily	2	2	0	4
			Twice a day	1	0	0	1
			Once in 2 days	1	1	0	2
			Total	4	3	0	7
	Overhead tank water (3)	UG (3)	Daily	1	1	1	3
			Total	1	1	1	3
Total samples: *P. aeruginosa absent*				9	10	4	23
Present (59) (72%)	Distilled water (52) (72%)	UG (40) (64%)	Daily	4	5	3	12
			Once in 2 days	7	8	6	21
			Weekly	2	0	2	4
			Once in 4 days	1	1	1	3
			Total	14	14	12	40
		PG (12) (63%)	Daily	1	1	1	3
			Twice a day	2	3	3	8
			Once in 2 days	0	0	1	1
			Total	3	4	5	12
	Overhead tank water (7) (70%)	UG (7) (11%)	Daily	1	1	0	2
			Once in 3 days	1	1	0	2
			Weekly	0	2	1	3
			Total	2	4	1	7
Total *P. aeruginosa* isolated				19 (68%)	22 (69%)	18 (82%)	59 (72%)
Total DUWL water samples investigated				28	32	22	82

DISCUSSION

Dental unit waterlines are an integral part of dental surgery equipment, supplying water as a coolant, primarily for air turbine and ultrasonic scaler. The patient and the attending dental staff may inhale a fine spray of this water, as it splashes off the surface of the patient's mouth. The presence of biofilm in DUWLs is a universal problem and pathogens from patients and the dental clinic environment can be cultivated from biofilm removed from DUWLs. The waterlines of dental units remain a potential weakness in the control of infection in the dental practice, as they can easily become contaminated with both patient-derived and municipal water impurities (Franco *et al*., 2005).

A total of 82 DUWLs water samples from 34 dental clinics were screened for *P. aeruginosa* and 59 samples were contaminated. Out of these, the water sample from ultrasonic scaler showed maximum 82% contamination of *P. aeruginosa* then 3-in-1 syringe 68% and air rotor 69%. As the diameter and material of the tubing were same, the reason for this finding may be due to different water flow rates or by the fact that ultrasonic scaler is used more frequently than 3-in-1 syringe and air rotor in the clinics (Walker *et al*., 2000).

The dental clinics using distilled water 72 for flushing showed 72% (52) whereas clinics using overhead tank water showed 70% (7) *P. aeruginosa* contamination. The distilled water that was in use might be contaminated, addition of distilled water in residual water, improper as well as less frequency of cleaning the storage tank; all these factors might be contributed for the contamination or formation of biofilms containing of *P. aeruginosa*. Moreover it was observed that the dentist's with post graduate qualified dentist keeps dental water less 63% *P. aeruginosa* contaminated or free from biofilm formation as compared to dentists with undergraduates qualified dentist clinic 75%. The 59 (72%) isolates of *P. aeruginosa* were isolated from dental clinics where frequency of washing of water container was daily 53%, twice a day 89%, once in two days 79%, once in three days 100%, once in

Table 3: MARI of *P. aeruginosa* isolated from DUWL water

Antibiotics	Resistant isolates	Sensitive isolates	MAR index
Novobiocin	59	0	0.1250
Cefaclor	59	0	0.1250
Nitroflurantoin	58	1	0.1220
Erythromycin	58	1	0.1220
Piperacillin	15	44	0.0310
Gatifloxacin	13	46	0.0270
Ciprofloxacin	2	57	0.0040
Amikacin	1	58	0.0021

four days 100% and weekly 88%. The data indicated that the increase in frequency of washing of water container and water lines decreased the *P. aeruginosa* contamination and increase in the quality of DUWLs water (Table 2). Increase in washing frequency of water container and the flushing through of water lines between patient and at the beginning and end of the working day eliminates the bacterial contamination, which is a useful method to eliminate oral flora entering the waterline via suck-back. The dental water lines' *P. aeruginosa* showed 100% resistance to novobiocin and cefaclor followed by nitrofurantoin and erythromycin 98%, almost 100% sensitive to ciprofloxacin and amikacin and 75-78% sensitive to piperacillin and gatifloxacin. The MAR index of antibiotics against *P. aeruginosa* showed highest MAR index 0.125 against novobiocin and cefaclor followed by 0.122 for nitrofurantoin and erythromycin, respectively. Thus, these antibiotics should not be used against infection in dental clinics whereas amikacin (MARI 0.0021) and ciprofloxacin (MARI 0.004) can be drug of choice against *P. aeruginosa* in dental treatment (Table 3).

CONCLUSIONS

Since the origin of dental unit water contamination is now more clearly defined, dental manufacturers and the scientific community in approaches can make substantial progress to prevention and control. More contamination of *P. aeruginosa* was found in water samples due to the multiple ports of the entry to the DUW system for microbes, no single method or device will completely eliminate the potential for cross infection. The ultrasonic scalar showed highest contamination of *P. aeruginosa* than other two hand pieces (3-in-1 syringe and air rotor). Combinations of currently available procedures and equipment including anti-retraction devices, flushing, independent water supplies used in conjunction with biocide purges or fully autoclavable water line circuitry should provide water, which is of a higher standard than that of a drinking water. Sterile water or saline should be provided from a separate source, which cannot be contaminated by passage through the DUWLs. The dental clinics using distilled water as a source of water for treatment showed more *P. aeruginosa* contamination. Therefore chair side devices for monitoring microbial quality of the DUW need to be developed and are an essential component to satisfactory water quality. Existing recommendations for flushing through of water lines between patients and at the beginning and end of the day is a useful method to eliminate oral flora entering the waterlines via suck-back. All these systems required strict adherence to maintenance protocols to perform to their full potential.

REFERENCES

Al-Hiyasat, A.S., S.Y. Ma'ayeh, M.Y. Hindiyeh and Y.S. Khander, 2007. The presence of *Pseudomonas aeruginosa* in the dental unit waterline systems of teaching clinics. Int. J. Dent. Hyg., 5: 36-44.

Anonymous, 2000. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grows Aerobically. Approved Standard M7-A5 National Committee for Clinical Laboratory Standards, Wayne, PA.

Bauer, A.W., W.M.M. Kirby and J.C. Sherris, 1966. Antibiotic susceptibility testing by a single disc method. Am. J. Pathol., 45: 493-496.

Collee, J.G., A.G. Frasier, B.P. Marmion and A. Simmons, 1996. In Mackie and McCartney's Practical Microbiology. 14th Edn., Churchill Livingston, New York, pp: 978.

Forde, A., P. O'Reilly and G.O. Fitzgerald *et al*., 2005. Microbial contamination of dental unit water systems. J. Ire. Dent. Assoc., 51: 115-118.

Franco, F.F.S., D. Spratt, J.C. Leao and S.R. Porter, 2005. Biofilm formation and control in dental unit waterlines. Biofilms, 2: 9-17.

Krumperman, P.H., 1983. Multiple antibiotics resistance indexing of *Escherichia coli* to identify risk sources of fecal contamination of foods. Can. J. Microbiol., 46: 165-170.

Martin, M.V., 1987. The significance of the bacterial contamination of dental unit water systems. Br. Dent. J., 163: 152-153.

Mavridou, A., J. Kamma, G. Mandilara, P. Delaportas and F. Komioti, 2006. Microbial risk assessment of dental unit water systems in general dental practice in Greece. Water Sci. Technol., 54: 269-273.

Monteiro de Souza-Gugelmin, M.C., C.D. Torre Lima, S.N. Marques de Lima, H. Mian and I.Y. Ito, 2003. Microbial Contamination in Dental Unit Waterlines. Braz. Dent. J., 14: 55-57.

Pankhurst, C.L., W. Coulter, J.J. Philpott-Howard, T. Harrison, F. Warburton and S. Platt, 2003. Prevalence of Legionella waterline contamination and *Legionella pneumophila* antibodies in general dental practitioners in London and rural Northern Ireland. Br. Dent. J., 195: 591-594.

Rautemaa, R., A. Nordberg, K. Wuolijoki-Saaristo and J.H. Meurman, 2006. Bacterial aerosols in dental practice-a potential hospital infection problem? J. Hosp. Infect., 64: 76-81.

Walker, J.T., D.J. Bradshaw, A.M. Bennett, M.R. Fulford, M.V. Martin and P.D. Marsh, 2000. Microbial Biofilm Formation and Contamination of Dental-Unit Water Systems in General Dental Practice. Applied Environ. Microbiol., 66: 3363-3367.

Walker, J.T., D.J. Bradshaw and M. Finney *et al*., 2004. Microbiological evaluation of dental unit water systems in general dental practice in Europe. Eur. J. Oral. Sci., 112: 412-418.