International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:4, No:6, 2010

Assessment of Microbial Pollution of the Dental Chairs Water System (*Pseudomonas aeruginosa)* in the City of Tripoli, Libya

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***Abstract*—**This study mainly aims at assessing the level of microbial pollution of the water used in the chair system in dental clinics. For this purpose 36 samples have been randomly collected from a number of dental surgeries in the city of Tripoli in Libya. However, 32 of the samples have tested positive to microbial pollution including 13 of the samples, which have tested positives to *Pseudomonas aeruginosa*. Based on the results of the test a further investigation of the biofilms incorporated within the dental chair system has been conducted. The laboratory tests of biofilms with similar design to those found in dental chairs have proved that bacterial pollution takes place through saliva of the patients who use the chairs, and that this saliva is rich with nutrients which provides a suitable breeding ground for all types of bacteria.

Keywords*—Pseudomonas aeruginosa*, Biofilm.

I. INTRODUCTION

THE bacteria in biofilms have several benefits; most importantly it is used for sewage treatment in biological decomposition stations. However, biofilms could cause medical problems as the case with dental chair water systemus. In other words, pollution of medical instruments with these bacteria could be a serious health hazard [9]. For example, *Pseudomonas aeruginosa* is an aerobic optional bacterium, which usually grows in wet places such as soil or any water laden habitat. It is also known to exist in biofilms [8]. *Pseudomonas aeruginosa* is described by experts as an opportunistic bacterium which usually affects both children and elderly people causing severe infection especially in relation to surgeries associated with internal and external sinuses [10]. However, statistics from the USA have showed that many incidents of infection in hospitals were associated with *Pseudomonas aeruginosa* at a rate of infection of 0.4 %. Yet, in a study featuring 121 samples of water from dental chair systems, the results proved bacterial contamination with *Pseudomonas aeruginosa* among the most common species [6].

II. MATERIALS AND METHODS

A. Study Area and Collection of Water Samples:

The study has been confined to the city of Tripoli. For convenience the city has been subdivided into four main areas which are: the city centre, Abu-Saleem, Suk-Aljumaa and Andalus-town. A total of 36 samples featuring dental chair water systems have been collected from 21 private and public dental clinics. The samples have been used to establish the total count of bacteria in general and the presence and count of *Psoudomonas aeruginosa*, and Coliform bacteria in particular (Table 1).

TABLE I SHOWING THE NUMBER OF AND LOCATION OF SAMPLES FEATURING DENTAL CHAIR WATER SYSTEMS FROM VARIOUS DENTAL CLINICS WITHIN THE CITY OF TRIPOLI

Area	Number of surgeries	Number of samples
City center	9	16
Abu-Sleem	3	3
Suk-Aljumaa	4	7
Andalus- town	5	10

B. Unit (Reactor) Design:

Three laboratory units similar to the dental chair water systems have been designed. Each unit is made up of a seven-litre tank made of PVC material. A plastic tube is connected to the bottom of the tank and a valve controls the flow of water from one direction i.e. out of the unit. Each unit has been supplied with 6 litre of water contaminated with *Psoudomonas aeruginosa* together with certain nutrients (Figure 1, and Table 1).

TABLE II SHOWING THE VARIOUS TYPES OF NUTRIENTS THAT HAVE BEEN ADDED TO THE UNITS FOR STUDYING BIOFILM

Unit (Reactor)	Type of nutrients
Unit 1	Without any nutrients
Unit 2*	K_2HPO_4 (18g/6l), $MgSO_4$ (1.2g/6l), $CaCl_2$ (0.6g/6l), $FeSO_4$ (0.006g/6l) $C_6H_{12}O_6$ (6.0g/6l)
Unit 3**	Saliva

*Was add in three time (during 21 days).
**Was add every day (during 21 days).

C. Collection of Water and Biofilm Samples from Reactors:

Samples of water were gathered from the three units. The sampling process continued for 21 days at the rate of 2 samples from each tank (one from the top and the other from the bottom) every three days. The plastic tubes were also disconnected from the tanks and the inside walls of the tube were examined for the formation of the thin bands (Biofilms).

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Samples were collected from the bands. All the samples were then examined for *Psoudomonas aeruginosa.*

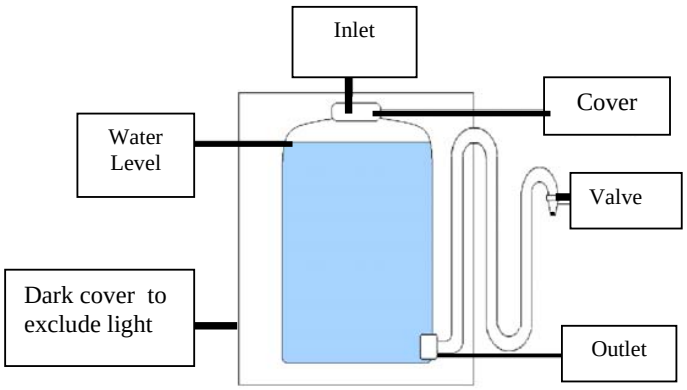

Fig. I A diagram showing a section of a laboratory unit mimicking the dental water systems.

C. Analytical Techniques

A.Total Bacterial Count

The total number of bacteria was made by the standard plate method. By using Plate Count Agar as a nutrient, and incubation at a temperature of 37° C for 24 hours the numbers of bacterial growth were known by direct counting [1].

B. Total Coliform Bacteria Count

The estimation was made through membranous filtration, by using the suitable nutrient for bacterial growth, known as (M-Endo Brout Amplues). The system was incubated for 24 hours at a temperature of 35° C, and the numbers of bacterial growth were known by direct counting [1].

C. Quantification of Psoudomonas aeruginosa Bacteria

The above method was used with a different nutrient medium known as (Psoudomonas Broth Ampules), with 24 hour incubation at 35° C. The number of bacterial growth was known by direct counting [1].

D. Absolute Quantification of Psoudomonas aeruginosa Bacteria

E. Test One (At 37 ° C)

The tests involve incubation at 42° C, and using the same method as shown above [1].

F. Test Two (Oxidation Test):

This test involved the isolation of some colonies of *Psoudomonas aeruginosa* bacteria, which were identified by the above shown method. A culture of the bacteria was made by using Agar nutrient and 24 hours incubation at 42°C. Then some drops of TMDD solution were added to the filter paper in a standard plate. Some bacterial colonies which formed at the beginning of this test were to be transferred to the filter paper, whereby the purple colour would confirm the presence of *Psoudomonas aeruginosa* [1].

II. Results and Discussion

A. The total bacterial count in the water of the dental chair system

Table 3 shows the results of samples which have tested positive (microbial content) in terms of total bacteria count. Of the total number of samples which have been collected for the study; 19 samples have tested positive to bacteria (table 1) while the rest of the samples are bacteria-free. The highest bacterial count in the samples was 3.6 x 10^4 cells / ml while the lowest bacterial count was 520 cells / ml. These figures exceed the permissible local and international limits. According to the EU specifications the microbial count in dental chair water should not exceed 100 cells / ml, while the American Society for Dentists increases that limit to 200 cell /ml [3]. In Libya however, the specifications for drinking water can be taken as a guide, which indicate that the bacterial count should not exceed 500 cell / ml (The Libyan Specification Office, 2008). Yet, as far as this study is concerned the bacterial count exceeds both limits set by the EU and the American Dental Society by 66 % and 61 % in the total number of samples respectively. The bacterial count provides a good indicator to the effectiveness of sterilisation and disinfection processes featuring the different parts of the dental chair systems particularly those parts which are associated with water in the system. So, the closer the results of the test to the specified limits, the more efficient the sterilisation and disinfection processes will be and vice versa. The results of the current study seem to be consistent with the results of a study featuring seven European countries in which the results that have been obtained have exceeded the permissible limit by 51 % [3].

TABLE III THE TOTAL BACTERIAL COUNT IN CONTAMINATED SAMPLES

Area	Sample	Number of total count bacteria Cell/ml
City center	1	9.0 X 10^2
	2	3.0 X 10^3
	3`	2.3 X 10^3
	4	1.3 X 10^4
	5	5.1 X 10^3
	6	2.1 X 10^4
	7	1.4 X 10^3
	8	1.8 X 10^3
	9	1.2 X 10^4
	10	3.0 X 10^4
	11	5.2 X 10^2
Andalus- town.	12	1.1 X 10^4
	13	5.4 X 10^3
	14	2.8 X 10^4
	15	3.6 X 10^4
	16	2.3 X 10^3
Suk-Aljumaa	17	1.8 X 10^3
	18	9.5 X 10^2
Abu-Sleem	19	2.0 X 10^3

B. The number of Pdeudomonas aeruginosa in the Dental Chair water samples:

Table 4 shows the number of *Pdeudomonas aeruginosa*. However, only 13 samples of the total samples have tested positive to *Pdeudomonas aeruginosa* (table 1) while the rest of the samples have tested negative to the microbe. The highest value was (12 x 10^2 cell / 100 ml), while the lowest value was (0.03 x 10^2 cell / 100ml). As far as the Dental chair water specification is concerned this type of bacteria is deemed harmless, and yet with regard to drinking water particularly mineral water in jars it is recommended that it must be free of this type of bacteria i.e. (0 cell / 100 ml).

Regarding the results of this study the levels have exceeded the permissible limit by 36.1 % in the total samples that have been investigated. Pollution with this type of bacteria takes place through the patients' saliva which leaks into the water of the dental chair system as the system is poorly cared for and not hygienically suitable for use. Again the results obtained in this study seem to be consistent with the results featuring Parma hospital in Italy whereby the results of the tests of the dental chair water samples have indicated that 41.1 % of the samples exceed the permissible limit in terms of its *Pdeudomonas aeruginosa* bacteria count. [7].

TABLE IV THE NUMBER OF *PDEUDOMONAS AERUGINOSA* IN CONTAMINATED SAMPLES

Area	Sample	Number of *Pdeudomonas aeruginosa* bacteria Cell/100ml
City center	1	1.5 X 10
	2	7.2 X 10^2
	3	12 X 10^2
	4	4.8 X 10^2
	5	0.3 X 10
	6	1.2 X 10^2
Andalus town.	7	1.8 X 10^2
	8	1.5 X 10^2
Suk-Aljumaa	9	2.4 X 10^2
	10	4.0 X 10
	11	3.6 X 10^2
Abu-Sleem	12	1.2 X 10^2
	13	2.4 X 10^2

C. Confirmation tests for the Pdeudomonas aeruginosa bacteria count in contaminated samples:

Table 5 shows the results of the confirmatory tests for *Pdeudomonas aeruginosa* bacteria count, which has been estimated, incubated at 37° c and finally grown at 42° c. The oxidez test has been positive showing the distinctive purple colour for all samples.

TABLE V THE CONFIRMATORY TESTS FOR *PDEUDOMONAS AERUGINOSA* COUNT IN WATER SAMPLES OF THE DENTAL CHAIR SYSTEMS

Area	Sample	*Pdeudomonas aeruginosa* bacteria at 37 ^{O}C	*Pdeudomonas aeruginosa* bacteria at 42 ^{O}C
City center	1	+	+
	2	+	+
	3	+	+
	4	+	+
	5	+	+
	6	+	+
Andalus-town.	7	+	+
	8	+	+
Suk-Aljumaa	9	+	+
	10	+	+
	11	+	+
Abu-Sleem	12	+	+
	13	+	+

D. Estimation of Pdeudomonas aeruginosa bacteria in the units designed for the purpose of this study:

From Table 6 it becomes obvious that the *Pdeudomonas aeruginosa* bacteria count has decreased in the first unit as no nutrients have been added to the units (table 2) after 21 days from the beginning of the experiment. In the second unit however, some nutrients have been added as shown on table (2). As a result the number of the same bacteria has increased significantly during the same period of time as indicated in the first unit. But nonetheless, as for the third unit the increase has been even more significant than in the second unit due to addition of saliva as a nutrient. This proves beyond doubt that saliva constitutes a main source of contamination in case of the dental chair systems as the *Pdeudomonas aeruginosa* count increases dramatically with the increase of salivary fluids in the system, which contains the essential nutrients for the growth of this type of bacteria. In this regard studies that have been conducted by Arrbty [2] and Fadel [5] have proved that saliva contains all the essential ingredients for bacterial growth.

TABLE VI *PDEUDOMONAS AERUGINOSA* BACTERIA COUNT IN THE UNITS DESIGNED FOR THE PURPOSE OF THIS STUDY

Time (days)	Number of *Pdeudomonas aeruginosa* bacteria (Without any nutrients) Cell/100ml	Number of *Pdeudomonas aeruginosa* bacteria + nutrients Cell/100ml	Number of *Pdeudomonas aeruginosa* bacteria + Saliva Cell/100ml
3	38 X 10^2	12 X 10^2	52 X 10^2
6	13 X 10^2	5.0 X 10^2	25 X 10^2
9	19 X 10^2	9.0 X 10^2	9.0 X 10^2
12	20 X 10^2	9.0 X 10^2	27 X 10^2
15	18 X 10^2	9.0 X 10^2	19 X 10^2
18	13 X 10^2	11 X 10^2	66 X 10^2
21	10 X 10^2	15 X 10^2	90 X 10^2

E. The bacterial count of the biofilms of the units:

From the samples that have been collected from the biofilms featuring the third unit which has been supplied with salivary nutrients, the maximum bacteria count has been 1500 cells / 100ml on average. The samples have been collected from the bottom surface of the units, while the biofilm samples have been collected from the inner walls of the tubes of the third unit with bacterial count of 200 cell / 100 ml on average.

III. Conclusion

The study has proved that the water of the dental chair system in some dental clinics has been contaminated with *Pdeudomonas aeruginosa* bacteria. The main reason behind this contamination is that these chair systems are not operating properly; whereby nutrient-rich salivary fluids from patients leak into the water in the system providing a suitable breeding ground for bacteria.

References

[1] American Public Health Association (APHA). (1995) Standard methods for Examination of Water and Wastewater. 19thEd, Washington, D. C.

[2] Arraby, A. S. (1999). Texbook of Oral Microbiology. Tripoli-Libya(1):67-68.

[3] Chate, A. R. (2006). An audit Improve. The Quality of Water within The Dental Unit Water Lines of Three Separate Facilities of a United Kingdom NHS Trust. *British Dental Journal* (201):265-269.

[4] Center Nation Libya for Measure (CNLM). (2008) Libya Specifics No (83). Tripoli-Libya.

[5] Fadel. M. (2009). Oral Histolog and Embryolog, (3rd) edition. Tripoli-Libya.

[6] Jean, B., Richard, T., Esther, F., Catherine, A., Luc, T. and Luger, P. P. (1996). Multiparametric Analysis of Waterline Contamination in Dental Units, *Applied and Environmental Microbiology, 11(62):3954-3959.*

[7] O'Donnell , D . J ., Tuttle , C. M ., Falkiner, F . R . and Coleman, D .C.(2005) Bacterial Contamination of Dental Chair Units in A modern Dental Hospital Caused By Leakage From Suction System Hoses Contaning Extensive Biofilm , *Journal of Hospital Infection* ,59 (4) : 348-360 .

[8] Todar, K. (2008) Bacteriology (Pesudomons aeruginosa). Press University of Wisconsin-Madison, Department of) Bacteriology, USA.

[9] Paula, H. D. (2003) Biofilm. 2th Ed., Edstron Industries, New York,USA.

[10] Qarah, S. and Cunha. A, B. (2005). *Pseudomonas aeruginosa* Infection. *E, Medicne, (12):pp1-10.*