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Original Research Article.

Bacterial Contamination of Water in Surgery Dental Units

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ABSTRACT

Background: The water obtained from dental units via syringes, air rotors, low-speed handpieces, tube surfaces and water cup filler may be a potential source of infection and antimicrobial resistant microbes in the dental unit water (DUW) systems for both practice staff and patients.

Aim and Method: To assess the quality and quantity of microbial contamination using the membrane filter method, determine the antibiotic susceptibility and the minimum inhibitory concentration (MIC) from different points (the syringe, air rotor, water cup filler and water main source) in ten surgeries dental clinics (DCs) by using the VITEK 2 system and E-test.

Results: The quantity of viable count on plate count agar was 30 – 304 CFU/ml, while the *Pseudomonas aeruginosa*, coliform bacteria and *Candida albicans* were 0 CFU/100ml. The difference between the water samples in the present study were not statistically significant and gave *P* value 0.277. The quantity of identified Gram-positive cocci bacterial isolates includes *Micrococcus luteus* 50.1%, *Micrococcus lylae* 46.2% and *Staphylococcus hominis* 1%, while the Gram negative bacterial isolates include *Sphingomonas paucimobilis* 2%, *Pantoea agglomerans* 0.2%, *Francisella tularensis* 0.1% and *Acinetobacter lwoffii* 0.2%. The Gram positive cocci isolates were sensitive to Clindamycin, Erythromycin, Gentamicin, Levofloxacin, Linezolid, Moxifloxacin, Nitrofurantoin, Oxacillin, Teicoplanin, Tigecycline, Tobramycin, Trimethoprim/ Sulfamethoxazole and Vancomycin, while the Gram negative bacteria were sensitive to Amikacin, Imipenem, Tigecycline and Trimethoprim/Sulfamethoxazole.

Conclusion: The microbiological monitoring water quality should be followed. Using a treated main source of water is important and the filtration method in dental clinics is an effective mechanism to reduce the microbial contamination in DUW and reduction the biofilm formation in general dental practice.

Key words: Dental Surgery Unit, Water Contamination, Antimicrobial Resistant, VITEK 2 System, E-Test.

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INTRODUCTION

Dental units are complex operating systems that provide air and water to equipment on the unit during patient treatment due to direct transport the water from the municipal water supply to the dental unit system. The dental unit water line monitoring is essential for the safety of patients and dental healthcare staff during treatment.[1,2]

Bacterial biofilm in DUW lines is a widespread problem, biofilms are defined as structured communities of microorganisms that are attached to each other and to surface, embedded in a protective matrix.[3,4] Many oral infectious diseases occurred due to a dynamic interaction between microorganisms, their host, and the host's diet, leading to microbial colonization and the pathogenic biofilms formation on oral surfaces.[5,6] Bacterial biofilm can begin forming in a new dental unit within a few days that dependent on the species composition, microbial metabolism and environmental conditions.

The dental water biofilm is composed of different bacterial species which produce extracellular polysaccharides that bind the bacterial cells together, replicated on the tubing surface in any aquatic system and become very difficult to remove once formed.[7-9]

To reduce the accumulation of biofilm in the dental water supply the Australian Dental Association (ADA) was recommending (i) the monitoring water quality is necessary, maintained regularly and using the appropriate methods to maintain the recommended quality by using ozonation or electrochemical activation, chemical dosing of water (e.g. with hydrogen peroxide, peroxygen compounds, silver ions, or nanoparticle silver), filters installed near the handpieces in dental water is very important, (ii) the microbial count standard of dental treatment water should be less than 500 CFU/mL in the drinking water, (iii) flushing waterlines at the start of the day and after each patient uses to weekend biofilm

accumulation, (iv) all waterlines must be fitted with non-return (anti-retraction) valves to help prevent retrograde contamination of the lines by fluids from the oral cavity.[10-14] Antimicrobial resistance of microorganisms isolated from DUW is a result of several factors such as binding of the agent, a lack of penetration of inhibitors, the localization of neutralizing enzymes, the low growth rate of the microbes, and the expression of a resistant phenotype due to surface growth.[15] All of this led us to focus our research on investigate the microbial contamination levels and detect the pathogens and the antibiotic susceptibility of pathogenic isolates from dental unit waterlines in dental surgical practices.

MATERIALS AND METHODS

This study assessed the microbial contamination quality, quantity, detecting the present coliform, *P. aeruginosa* and *C. albicans*, determine the antibiotic susceptibility and MIC of identified microbial isolates in water samples taken from surgery Dental Teaching Hospital, Umm Al-Qura University, Makkah, Saudi Arabia.

Collection of Water Samples

Samples were taken from four different points in each DUW system during the surgical dental clinics: (i) the syringe, (ii) the air rotor, (iii) the water cup filler, (iv) water main source. DUW samples were shown in surgery DC layout Figure 1. One-hundred millilitres of water was collected from a sterile nozzle into a sterile water container (Saudiplast, KSA) and immediately transported to the laboratory in the insulated ice box to complete the microbiological analysis.

Viable Counts onto Nonselective Media

After collecting the water samples, 100ml was filtrated through 45µ, 47mm diameter membrane and sterile filter paper (Millipore, white, Mixed Cellulose Esters) analytical glass vacuum filter holder 300mL capacity and 47mm diameter (Millipore, USA) in aseptic condition to collect the waterborne microorganisms. The filter membrane was cultured on Plate Count Agar in aseptic condition and incubated aerobically at 37°C for up to 48 hours. The viable counts were evaluated by using the membrane filter method as described by Momeni et al., 2012.[16] The European Union standards for potable water and the American Dental Association standards of aerobic bacteria in DUW system should be ≤500 CFU/mL.

Viable Counts onto Selective Media:

To perform a risk analysis for pathogens in drinking-water, it is necessary, to study the interactions between total viable bacterial count and total pathogens include coliform, *P. aeruginosa* and *C. albicans* isolates. They enumerated the coliform, *P. aeruginosa* and *C. albicans* using the membrane filtration technique according to Momeni et al., 2012[16] on MacConkey agar, Pseudomonas agar and Sabouraud Dextrose Agar (Oxoid, UK) respectively in triplicate. The European Union standard for potable water and the American Dental Association standards of coliform, *P. aeruginosa* and *C. albicans* in DUW system should be 0 CFU/100mL.

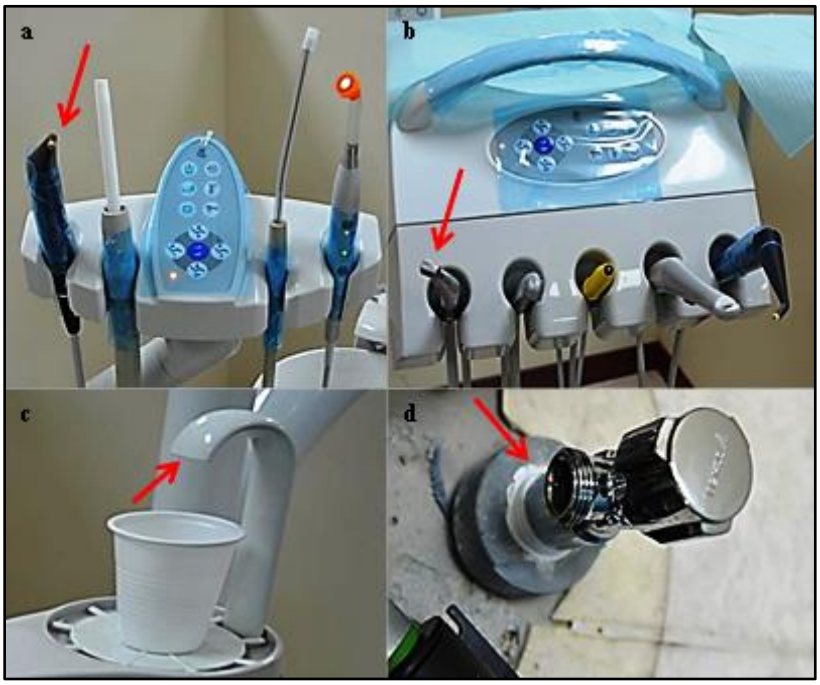


Figure 1: The surgery DC water sources in the present study.
(a) syringe, (b) air rotor, (c) water cup filler and (d) water main source.

Identification of Bacterial Isolates

The bacterial isolates were identified by morphological, biochemical characteristics by VITEK 2 system.

Susceptibility to Antibiotics

The antibiotics susceptibility and MIC were determined by using the VITEK 2 system. While the unknown antibiotics susceptibility and MIC by VITEK 2 system were determined by using disk diffusion methods and E-test (BioMérieux) respectively according to Clinical Laboratory Standards Institute 2013.[17] All tests were performed in Mueller–Hinton Agar (Oxoid, UK) in triplicate.

Statistical Analysis

The mean values, SD, minimum, maximum interval of CFU/ml were calculated. The statistical test used analysis of variance (ANOVA). Differences between the results from each water source were investigated by comparing the means using the Tukey multiple comparisons test. Statistical analysis was carried out using portable SPSS statistics version 19.

Ethical Disclosures

The authors announce that no experiments were performed on voluntaries or animals and no data were collected from the patients in this research. The authors have obtained the written approval of the Teaching Dental Hospital, Umm Al-Qura University, Makkah, Saudi Arabia to do this study.

RESULTS

This part includes the results of this study that were obtained from surgery DUW system, investigated the microbial contamination levels and the pathogen isolates (coliform, *P. aeruginosa* and *C. albicans*). Also determined the antibiotics susceptibility and MIC value of identified isolates from dental unit waterlines. This study was performed in the surgery department of the Teaching Dental Hospital, Umm Al-Qura University, Makkah, Saudi Arabia.

Microbiological Quality of Water

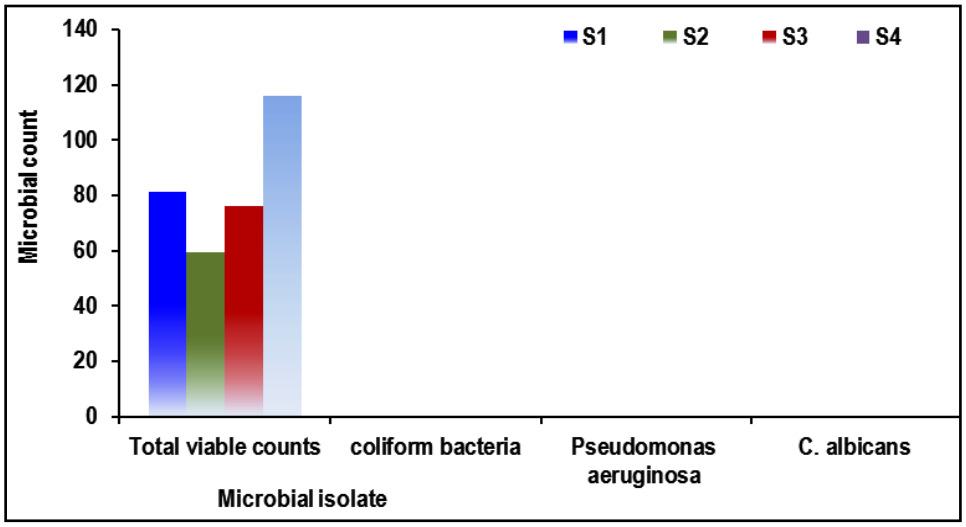
The mean, standard deviation, maximum and minimum of CFU/ml in different surgery DC water samples were cultured on Plate Count Agar in aseptic condition recorded in Table 1, all the results were present within the limit according to the European Union standard for potable water and the American Dental Association standards for DUW system.

The mean and standard division of the total viable counts in water cup filler, air rotor, syringe and water main source sample were 81.4 / ±66.017, 59.4 / ±43.757, 76.0 / ±55.233 and 115.8/ ±86.335, while the minimum/ maximum results gave 34/208, 30/180, 31/194 and 38/304 CFU/ml respectively. The mean viable counts of coliform and *Pseudomonas* isolates were present in the limit and gave 0 CFU/100ml in all water samples. All data was collected in Fig 2.

Table 1: Mean, standard deviation, maximum and minimum of the total viable counts in different surgery DC water samples on Plate Count Agar medium.

Total viable counts	Water source					*P* value
	S1	S2	S3	S4	Total	
Mean/ ± SD	81.4 / ±66.017	59.4 / ±43.757	76.0 / ±55.233	115.8 / ±86.335	83.15 / ±65.602	0.277
Minimum	34	30	31	38	30	
Maximum	208	180	194	304	304	

S1= water cup filler, S2= air rotor, S3= syringe and S4= water main source.



S1= water cup filler, S2= air rotor, S3= syringe and S4= water main source.

Figure 2: Interactions between the mean of total viable bacterial count and pathogens (coliform, *P. aeruginosa* and *C. albicans*) isolates in different dental surgeries water samples.

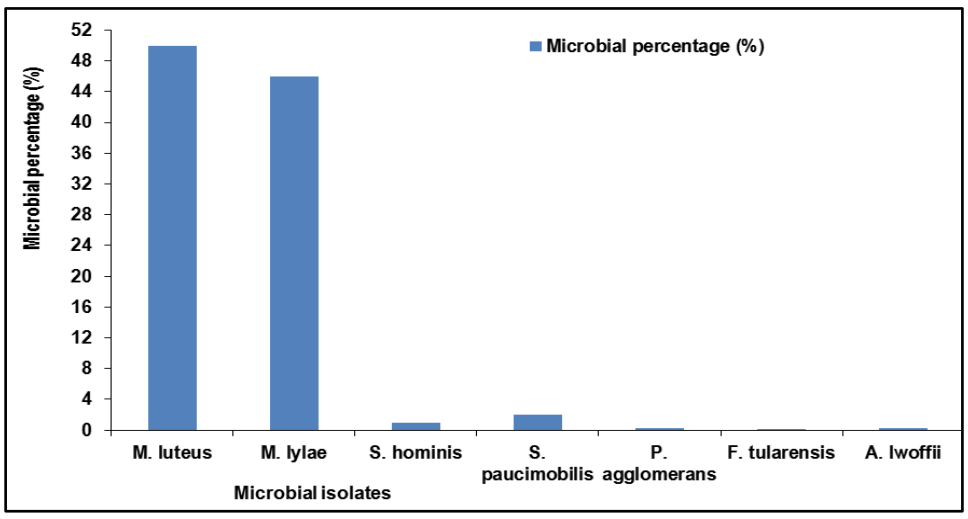


Figure 3: Microbial identification and percentage in DUW system by using the membrane filter method.

M. luteus, *M. lylae* and *S. hominis* were isolated from water cup filler, air rotor, syringe and water main source in surgery DCs, while the *Francisella tularensis* was isolated from syringe water sample, *Pantoea agglomerans* and *Acinetobacter lwoffii* were isolated from air rotor water samples and *Sphingomonas paucimobilis* was isolated from water cup filler water sample.

The quantity of *M. luteus*, *M. lylae*, *S. hominis*, *Sphingomonas paucimobilis*, *Pantoea agglomerans*, *Francisella tularensis* and *Acinetobacter lwoffii* isolates formed were 50.1, 46.2, 1, 2, 0.2, 0.1 and 0.2% respectively. All isolates were represented in Figure 3.

The antimicrobials were tested by VITEK 2 card against, *M. luteus* and *M. lylae* gave sensitive/MIC to Benzylpenicillin/<=2, Cefoxitin/<=2, Erythromycin/<=0.25, Gentamicin/<=8, Oxacillin/<=2, Levofloxacin/<=64, Linezolid/<=2, Moxifloxacin/<=2, Nitrofurantoin/<=16, Teicoplanin/<=1, Tetracycline/<=0.5, Tigecycline/<=16, Tobramycin/<=4, Vancomycin/<=0.25 and Trimethoprim/Sulfamet/<=10, while are intermediate sensitive/MIC to Rifampicin/<=0.5 and resistant/MIC to Fosfomycin/>=128 and Fusidic acid/>=32. While *S. hominis* gave sensitive/MIC to Clindamycin/<=0.25, Erythromycin/<=0.25, Gentamicin/<=0.5, Oxacillin/<=0.25, Levofloxacin/<=0.12, Linezolid/<=4, Moxifloxacin/<=0.25, Nitrofurantoin/<=16, Teicoplanin/<=0.5, Tigecycline/<=0.12, Tobramycin/<=1, Vancomycin/<=0.5 and Trimethoprim/Sulfamet/<=10, while are intermediate sensitive/MIC to Rifampicin/<=0.5 and resistant/MIC to Benzylpenicillin/>=0.5, Fusidic acid/>=32, Fosfomycin/>=128 and Tetracycline/>=16.

Table 2: MIC values and interpretation of isolated and identified bacterial species from surgery DCs water samples by using VITEK 2 system.

Antimicrobial	Bacterial isolates				
	S. paucimobilis	*S. hominis*	*M. luteus*	*M. lylae*	*A. lwoffii*
	MIC/In	MIC/In	MIC/In	MIC/In	MIC/In
Negative control	—	—	—	—	—
Amikacin	<= 2/S	—	—	—	<= 2/S
Amoxicillin/Clavulanic acid	<= 2/S	—	—	—	—
Ampicillin	<= 2/S	—	—	—	—
Ampicillin/Sulbactam	—	—	—	—	<= 2/S
Aztreonam	—	—	—	—	>= 64/R
Benzylpenicillin	—	>= 0.5/R	<= 2/S	<= 2/S	—
Cefepime	8/S	—	—	—	2/S
Cefoxitin	<= 4/S	—	<= 2/S	<= 2/S	—
Ceftazidime	>= 64/R	—	—	—	16/I
Ceftriaxone	2/S	—	—	—	—
Ciprofloxacin	<= 0.25/S	—	—	—	<= 0.25/S
Clindamycin	—	<= 0.25/S	1/S	1/S	—
Colistin	—	—	—	—	2/S
Erythromycin	—	<= 0.25/S	<= 0.25/S	<= 0.25/S	—
Fosfomycin	—	>= 128/R	>= 128/R	>= 128/R	—
Fusidic acid	—	>= 32/R	>= 32/R	>= 32/R	—
Imipenem	<= 0.25/S	—	—	—	<= 0.25/S
Gentamicin	<= 1/S	<= 0.5/S	8/S	8/S	<= 1/S
Levofloxacin	—	<= 0.12/S	<= 64/S	<= 64/S	0.5/S
Linezolid	—	4/S	<= 2/S	<= 2/S	—
Meropenem	1/S	—	—	—	0.5/S
Minocycline	—	—	—	—	<= 1/S
Moxifloxacin	—	<= 0.25/S	2/S	2/S	—
Nitrofurantoin	<= 16/S	<= 16/S	<= 16/S	<= 16/S	—
Oxacillin	—	<= 0.25/S	<= 2/S	<= 2/S	—
Piperacillin/Tazobactam	8/S	—	—	—	8/S
Rifampicin	—	<= 0.5/I	<= 0.5/I	<= 0.5/I	—
Tigecycline	<= 0.5/S	<= 0.12/S	<= 16/S	<= 16/S	<= 0.5/S
Teicoplanin	—	<= 0.5/S	<= 1/S	<= 1/S	—
Tetracycline	—	>= 16/R	<= 0.5/S	<= 0.5/S	—
Tobramycin	—	<= 1/S	<= 4/S	<= 4/S	<= 1/S
Vancomycin	—	<= 0.5/S	<= 0.25/S	<= 0.25/S	—
Trimethoprim/Sulfamethoxazole	<= 20/S	<= 10/S	<= 10/S	<= 10/S	<= 20/S

The MIC by microgram per milliliters. The numeric portion of the MIC is specific for that antibiotics and does not represent a relationship between the potential efficacy of one antibiotics over another. S= sensitive, I= intermediate, R= resistant.

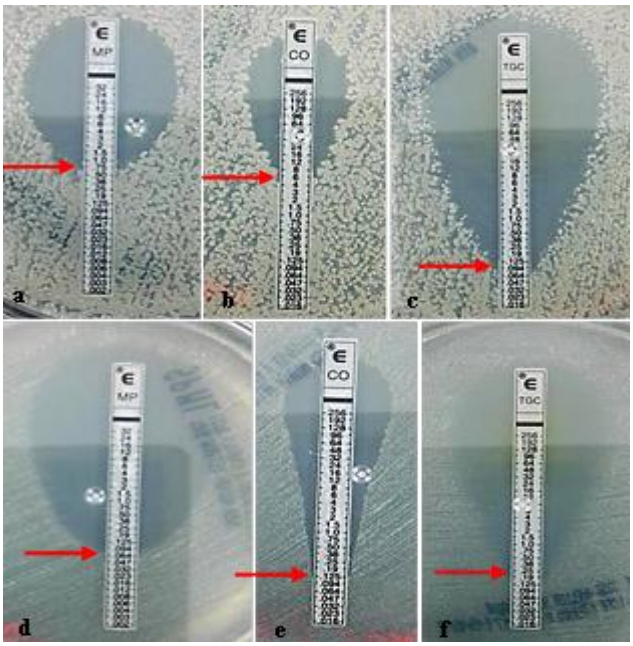
On the other hand, *Sphingomonas paucimobilis* gave sensitive/MIC to Amikacin/<=2, Amoxicillin/clavulanic acid/<=2, Ampicillin/<=2, Cefepime/<=8, Cefoxitin/<=4, Ceftriaxone/<=2, Ciprofloxacin/<=0.25, Gentamicin/<=1, Imipenem/<=0.25, Meropenem/<=1, Nitrofurantoin/<=16, Piperacillin/ tazobactam/<=8, Tigecycline/<=0.5 and trimethoprim/ sulfamethoxazole/<=20, while resistant/MIC to Ceftazidime/>=64. While, *Acinetobacter lwoffii* gave sensitive/ MIC to Amikacin / <=2, Ampicillin/ Sulbactam/<=2, Cefepime/<=2, Ciprofloxacin/ <=0.25, Colistin/<=2, Gentamicin/<=1, Imipenem/<=0.25, Levofloxacin/<=0.5, Meropenem/<=0.5, Minocycline/<=1, Piperacillin/Tazobactam/<=8, Tigecycline/<=0.5, Tobramycin/<=1 and Trimethoprim/ Sulfamethoxazole/ <=20. While it is intermediate sensitive/ MIC to Ceftazidime/ 16 and resistant/ MIC to Aztreonam/ >=64. All data was presented in Table 2.

The unknown antibiotics susceptibility by VITEK 2 system was determined by using disk diffusion methods and the MIC was determined by using E-test. *F. tularensis* isolate was sensitive to Amikacin, Amoxacillin/Clavulanic Acid, Colistin, Imipenem, Tigecycline and Trimethoprim/Sulfamethoxazole, while is resistant to Ampicillin, Cefepime, Cefoxitin, Ceftriaxone, Ciprofloxacin and Doxycycline. *P. agglomerans* isolate was sensitive against Amikacin, Ciprofloxacin, Colistin, Doxycycline, Imipenem, Tigecycline and Trimethoprim/Sulfamethoxazole, while is resistant to Amoxacillin/Clavulanic Acid, Ampicillin, Cefepime, Cefoxitin and Ceftriaxone. All data was presented in Table 3.

Table 3: Antimicrobial sensitivity of *F. tularensis* and *P. agglomerans* isolates from surgery DCs water samples by using disk diffusion method.

Antimicrobial (µg)	Bacterial isolates	
	F. tularensis	*P. agglomerans*
	Interpretation of zone diameters (mm)	
Amikacin / 30	32 / S	23 / S
Amoxacillin/Clavulanic Acid / 30	40 / S	23 / R
Ampicillin / 10	0 / R	0 / R
Cefepime / 30	15 / R	25 / R
Cefoxitin / 30	20 / R	22 / R
Ceftriaxone / 30	26 / R	28 / R
Ciprofloxacin / 5	24 / R	32 / S
Colistin / 10	31 / S	42 / S
Doxycycline / 30	20 / R	30 / S
Imipenem / 10	40 / S	33 / S
Tigecycline / 15	32 / S	40 / S
Trimethoprim/Sulfamethoxazole / 1.25	40 / S	30 / S

S= sensitive, R= resistant.



IMP= Imipenem, CO= Colistin and TGC= Tigecycline.

Figure 4. MIC values against isolates from surgery DCs water samples, (a, b, c) *F. tularensis* and (d, e, f) *P. agglomerans* by using E-test.

Table 4: Mean of the MIC values against *F. tularensis* and *P. agglomerans* isolates from surgery DCs water samples by using E-test.

Bacterial isolates	Antimicrobial MIC (µg/ml)	
	F. tularensis	***P. agglomerans***
Imipenem	<=0.50	<=0.064
Colistin	<=4	<=0.0125
Tigecycline	<=0.064	<=0.19

F. tularensis and *P. agglomerans* gave unknown MIC and interpretation results when tested by VITEK 2 card, for that the MIC was determined by using disk diffusion method and determine the MIC by using E-test. MICs were in the following ranges: Colistin 0.016– 256 µg/ml, Imipenem 0.02–0.32 µg/ml, and Tigecycline 0.016– 256 µg/ml. The mean values of MICs for the Imipenem, Colistin and Tigecycline shown <=0.50, <=4 and <=0.064 µg/ml against *F. tularensis* isolate respectively, while <=0.064, <=0.0125 and <=0.19 µg/ml against *P. agglomerans* isolate respectively, all data was collected in Table 4 and shown in Figure 4**.**

DISCUSSION

This study emphasizes the DUW system in Dental Teaching Hospital in Makkah to monitoring the microbial contamination in the DUW system and highlights to prevent infections in general dental practice and to have a good source of water for patients and dental healthcare staff.

The results of the present work revealed that the predominant bacterial isolates from the DUW lines were *M. luteus* and *M. lylae*. This is consistent with the previous studies by Kadaifciler and Cotuk 2014 who isolated the *Micrococcus* sp from dental units[18], All other studies, Venkatesh et al., 2006, Szymańska and Sitkowska 2013, Messano et al., 2013 and Lachachi, 2014 reported the presence of *Micrococcus* sp in the dental unit waterlines samples.[2,19-21]

This study, provided a list of few bacterial isolates from DUW lines which included: *S. hominis, A. lwoffii, F. tularensis, P. agglomerans* and *S. paucimobilis*. This is consistent with the previous studies that isolates *S. hominis*, *A. lwoffii* and *S. paucimobilis* from the water reservoirs in the dental units by Szymańska 2007.[22] Also, Yabune et al., 2008 isolated *S. paucimobilis* from contaminated DUW lines in the Conservative Dentistry Clinic of Osaka University Dental Hospital.[23]

In our study, the bacterial isolates are *Staphylococcus hominis*, that were considered as normal flora of the oral cavity according to a previous study by Ohara-Nemoto 2008.[24] Szymańska 2007 suggested the presence of *S. hominis* in the dental unit reservoirs due to the cross infections and sucking back fluids from patients' oral cavities.[22]

A. lwoffii is Gram negative, coccobacilli, considered a normal flora of the oropharynx and skin in approximately 25% of the healthy individuals and found in different environmental sources. *A. lwoffii* is a potentially opportunistic pathogen in patients with impaired immune systems, and it has been identified as a cause of nosocomial infections like septicemia, pneumonia, meningitis, urinary tract infections, skin and wound infections.[25-26]

In a previous study done by Umezawa et al., 2015 the resistant *A. baumannii* ST219 was isolated from water systems in Tokai University hospital from the emergency intensive care unit.[27] Also, Yabune et al., 2008 isolated *A. haemolytics* from contaminated DUW lines in the Conservative Dentistry Clinic of Osaka University Dental Hospital.[23] On the other hand, Barbeau et al., 1996 isolated *A. calcoaceticus* from water samples collected from dental units at the dental school of University de Montreal.[28]

F. tularensis is Gram negative coccobacillus, nonmotile, non-sporeforming, aerobic. *F. tularensis* spreads through the bite of infected animals, direct contact with infected tissue or contaminated soil, inhalation of aerosolized organisms, and ingestion of contaminated meat or water.[29] *F. tularensis* was isolated from a dental case from a tularemia region in the faculty of medicine by Tunga et al., 2007.[30]

Pantoea species are Gram negative bacilli in the Enterobacteriaceae family, which are pathogenic to both plants and humans. *P. endogenous* was isolated by Kletke et al., 2014 from endophthalmitis in a systemically healthy patient.[31] Rolph et al., 2001 isolated *P. agglomerans* from root canals.[32]

It was not possible to define primary antimicrobial agents for all DUW system isolates due to the great variation of the susceptibility among these pathogens, but Gentamicin, Tigecycline and Trimethoprim/Sulfamethoxazole shown the broadest spectrum against *S. paucimobilis, S. hominis, M. luteus, M. lylae* and *A. lwoffii* isolates. On the other hand, the MIC for *F. tularensis* and *P. agglomerans* showed sensitivity to Imipenem, Colistin, Tigecycline and Trimethoprim/Sulfamethoxazole for both. The mean values of MICs for Imipenem, Colistin and Tigecycline against *F. tularensis* and *P. agglomerans* were given 0.50, 4, 0.064, 0.064, 0.0125 and 0.19 respectively. Uzel et al., 2008 was shown the broadest spectrum of meropenem and ofloxacin against *A. calcoaceticus* and *S. paucimobilis.*[33]

On the other hand, the *Bipolaris* sp is sensitive to Batrafen, Canasten, Flucoral, Fungican and Mycosat antifungal compounds. However, Szymańska 2006 detected using hydrogen peroxide caused a significant decrease both in the number of total fungi and individual fungal species in DUW line.[34]

CONCLUSIONS

The present study is the first prospective study of DUW system in surgery Dental Teaching Hospital in Makkah. It was carried out to determine the quality and quantity of DUW system contamination. Though the prevalent organisms in DUW system are *M. luteus* and *M. lylae* and present within limits accepted according to European Union standard for potable water and the American Dental Association standards. The CDC recommended using the appropriate filtration methods, an anti-retraction valve and dental chair design to prevent and reduce the DUW system contamination. However, the colonization and proliferation of many and varied species of microorganisms and the biofilm might occur. For that we recommend: i) maintenance of sterility of DUW lines; ii) water should be monitored according to CDC and ADA recommendation not only to count of viable but also for the presence of coliform, *P. aeruginosa* and *C. albicans*; iii) using an

appropriate disinfectant or a filtration device, flushing and drying of DUW system, using autoclavable or disposable water delivery systems and using an independent sterile water reservoir that bypasses the municipal water.

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