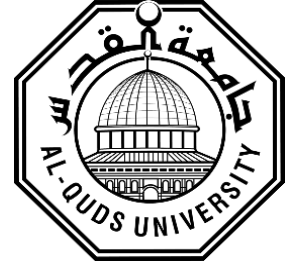


Deanship of Graduate Studies

Al-Quds University



Cultural and Molecular Evidence of *Legionella pneumophila* in Dental Unit Waterlines in the West Bank, Palestine

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M. Sc. Thesis

Jerusalem – Palestine

1441Hijri/ 2020AD

Cultural and Molecular Evidence of *Legionella pneumophila* in Dental Unit Waterlines in the West Bank, Palestine

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A thesis submitted in partial fulfillment of requirements for the degree of Master of Biochemistry and Molecular Biology / Faculty of Medicine -Al-Quds-University.

1441Hijri/2020AD

Dedication

To my mother and father...

To my dear brother, Mohammed...

To my sister, Maram...

To my family...

To my friends...

To my teachers...

To all the people who supported, and encouraged me.

Mutasem Zuheir Hilmi Burghal

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Thesis Approval

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Dental Unit Waterlines in the West Bank, Palestine**

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Declaration:

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed



Mutasem Burghal

Date: 19-1-2020

Acknowledgment

I am thankful to Allah for giving me the patience and strength to persevere through this endeavor. My gratitude and appreciation goes to every person who contributed and made this research work possible.

This research thesis was done under the supervision of **Dr. Dina M. Bitar** from the Faculty of Medicine, Microbiology and Immunology Department, Al-Quds University, Abu Deis, Jerusalem.

My deepest gratitude, appreciation, and respect are sent to my supervisor **Dr. Dina M. Bitar** for her enthusiasm, her inspiration and her great efforts to explain things clearly and simply. I am grateful for her endurance and for her editing suggestions and precise sense of language, which contributed to the final copy. Also, I want to thank her for giving me the opportunity to work on this project.

Miss. Suha Al- Butmeh, her guidance, professionalism, and help in the laboratory work, advice, support, patience, understanding and much more were key for getting me started and throughout this research project.

I want to express my deeply-felt and sincere gratitude to Al-Quds University, Department of Biochemistry and Molecular Biology represented by all the teachers and colleagues. I am deeply indebted to **Dr. Suhair Ereqat, Dr. Imad Matuq, and Dr. Kifaya Azmi**.

Also, let me thank and appreciate Microbiology Research Laboratory team **Dr. Ashraf Zayed, Mr. Mahmoud Amro and Mr. Ahmad Abdelkader** for their support, help, training, and valuable hints.

Also, I would like to thank the staff working at Al-Quds University.

Finally, I wish to thank my parents, my managers **Dr. Adnan Qarmash, Dr. Husam Tweir, Miss Fida Zeidan, Miss Najwa Essa** and my colleagues **Mahmoud Sapti, Maruf Noman and Adli Jarar** for providing a loving, tolerant and supportive environment. To them, I dedicate this thesis.

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Abstract

Legionella spp. is a Gram-negative, rod-shaped, a strictly aerobic and nutritionally fastidious bacterium. *Legionella pneumophila* is ubiquitous in aquatic environments and water distribution systems, including dental unit waterlines (DUWLs). Legionellosis is the disease caused by *Legionella* bacteria including Legionnaires' disease (LD), a fatal type of pneumonia, and the less acute form Pontiac fever, a flu-like illness. Among the 59 species and 70 serogroups of *Legionella spp.*, *L. pneumophila* is the major cause of sporadic and outbreak legionellosis (91.5%), and serogroup 1 is the predominant serotype (84.2%).

Many studies have demonstrated bacterial contamination of dental unit waterlines (DUWLs). When *Legionella* enters the DUWL from the main water reservoir, biofilms are formed on the inner surface of the waterlines. Biofilm provides suitable conditions for colonization and growth of *Legionella* within plumbing systems. Infection with *Legionella* occurs as a result of inhalation of aerosolized *Legionella* or aspiration of *Legionella* contaminated water by susceptible patients, health workers and dentists. The contamination of DUWLs with *Legionella* poses a serious health hazard for patients with chronic diseases and an impaired immune system.

Previous work in the Microbiology Research Laboratory performed a three-year (2012-2015) environmental surveillance of *Legionella* in the hospitals' water systems of eight hospitals across the West Bank. The study used culture and polymerase chain reaction (PCR) for the detection of *Legionella*. Their results showed low prevalence for *Legionella spp.* of 8.3% for water samples by culture, however this percentage increased to 50% by PCR. As for biofilms, The *Legionella* in biofilms was higher, being 16.8% by culture vs. 61% by PCR.

In this study we undertook to determine the prevalence of *Legionella* in water and biofilm samples from Tap and DUWLs collected from the dental clinics in the faculty of dentistry

at Al-Quds University (AQU) in Abu Deis Jerusalem and Arab American University in Jenin (AAUP), and dental clinics located in three major Palestinian cities; Nablus, Tulkarem, and Hebron in the West Bank.

The study samples included 185 samples, 89 (48%) water samples and 96 (52%) biofilm swabs, which were analyzed by cultivation dependent analysis (microbiological techniques) and by the cultivation-independent technique, namely PCR. For cultivation dependent analysis, the *Legionella* count was performed as well as serotyping of the isolates into serogroup 1 or serogroup 2-14. For cultivation-independent analysis, DNA was extracted from the samples and analyzed for the study of; the bacterial population, the presence of *Legionella* genus bacteria and for the presence of *L. pneumophila*, using 16S rRNA gene, Com, Lgsp, and L1 primers respectively. Partial sequencing of the 16S rRNA gene for seven *Legionella* isolates was done for further analysis for quality assurance and identification. Furthermore, water samples (Tap and DUWL) were tested for physical and chemical parameters. All samples were collected, processed and analyzed according to international standard operational procedures (SOPs) ISO 11731, ISO 11731-2.

L. pneumophila was isolated from 28 (15%) of 185 samples using cultivation dependent analysis and was detected in 142 (77%) of 185 samples using cultivation-independent analysis (PCR). PCR was 5x more sensitive than the culture technique, due to the Viable-But-Non-Culturable (VBNC) state of *L. pneumophila*. *L. pneumophila* was the only *Legionella spp.* that was detected in positive samples. *L. pneumophila* sg.1 was detected in 23/28 (82%) of the isolates, while 5/28 (18%) isolates were *L. pneumophila* sg. 2-14. All seven *Legionella* isolates' DNA sequenced for the 16SrRNA gene identified with *L. pneumophila* >95.7%. To ensure the quality of the water samples, their physical and chemical characteristics were measured; all were within acceptable ranges compared to WHO guidelines, except for carbonate hardness which was above WHO levels in 12 clinics and total hardness were above the WHO acceptable range in all clinics.

These results show that DUWLs of the examined dental clinics are contaminated with *L. pneumophila*. This finding reveals a serious potential health risk for infection of immunocompromised patients, health workers and dentists post-exposure.

The Ministry of Health (MOH) and the Palestinian Water Authority should put limitations and guidelines for water quality and microbiological monitoring, should advise washing of DUWLs with disinfectants such as chlorhexidine gluconate (CHX) or pure water and using

softener filters as well as routine periodic checking of DUWLs for bacterial contamination to ensure the health safety of patients and dentists.

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List of abbreviations

AE	Elution buffer
AP-PCR	Arbitrarily primed PCR
ACES	N-2-acetamino-2-aminoethansulfonic acid
AFLP	Amplified fragment length polymorphism
AQU	Al-Quds University
AAUP	Arab American University
ATS	American Thoracic Society
AW1	Wash buffers 1
AW2	Wash buffers 2
BTS	British Thoracic Society
BCYE	Buffered charcoal yeast extract
C	Celsius
CAP	Community-acquired pneumonia
CO₂	Carbon dioxide
CFU	Colony-forming unit
CHX	Chlorhexidine gluconate
DALY	Disability-adjusted life years
DNA	Deoxyribonucleic acid
Dot/icm	Defective organelle trafficking/ intracellular multiplication
DUWL	Dental unit waterlines
DCU	Dental chair unit
DW	Distilled water

ELB	Enzymatic lysis buffer
ELISA	Enzyme-linked immunosorbent assay
EtOH	Ethanol
EWGLI	The European working group for <i>Legionella</i> infection
GU	Genomic units
FDA	Fluorescent direct antibody
FISH	Fluorescent in situ hybridization
GVPC	Glycine Vancomycin Polymyxin B Cycloheximide
HCl	Hydrochloric acid
HIA	Health impact assessment
HPC	Heterotrophic plate count
HZI	Helmholtz Center for Infection Research
ICU	Intensive Care Unit
IDSA	Infectious Diseases Society of America
IFA	Immunofluorescence assay
ISO	International organization for standardization
KCl	Potassium chloride
KOH	Potassium hydroxide
L	Liter
LD	Legionnaires' disease
LLAPs	<i>Legionella</i> - like amoebal pathogens
M	Molar
mM	millimolar

M	Meter
MAb	Monoclonal antibody
Mbar	millibar
mg/ml	Milligram per milliliter
MIC	Minimal inhibitory concentration
Min	Minute
Mip	Macrophage infectivity potentiator
ml	Milliliter
MLST	Multi locus sequence typing
MLVA	Multi Locus Variable number of tandem repeat Assay
μl	Microleter
μm	Micrometer
μS	Micro Siemens
MOH	Ministry of health
NaCl	Sodium chloride
NAATs	Nucleic Acid Amplification Tests
Ng	Nanogram
ppm	Parts per million
PCR	Polymerase chain reaction
PVC	Polyvinyl Chloride
EPS	Extracellular polymeric substances
PFGE	Pulsed-field gel electrophoresis
PWA	The Palestinian Water Authority