

الجمهورية الجزائرية الديمقراطية
REPUBLICUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE
وزارة التعليم العالي و البحث العلمي

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE
SCIENTIFIQUE
جامعة 20 أوت 1955 - سكيكدة
UNIVERSITE 20 AOÛT 1955- SKIKDA



Faculté des Sciences

Département des Sciences de la Nature et de la Vie
Mémoire Présenté en Vue de l'Obtention du Diplôme de Master
Filière : Sciences Biologiques Option:
Microbiologie Appliquée

Intitulé

*"Screening And Identification Of Microorganisms
Responsible For Respiratory And Other Infections Originating
From Traditional Hookahs And Electronic Cigarettes."*

- Présenté Par :
- LANANI HAITEM
- BENLOUCIF MEROUA
- BALASKA HANA
- BOUDINAR ASMA

Membres de Jury:

Dr. BENDJAMA Abdallah	MCA	Président	Université 20 août 1955- Skikda
Dr. BECHEKER Imène	MCA	Directrice de mémoire	Université 20 août 1955- Skikda
Dr. GUEDDAH Doria	MCB	Examinatrice	Université 20 août 1955- Skikda

Année universitaire 2024/2025

Acknowledgements:

First and foremost, all praise and gratitude are due to **Allah**, the Most Gracious, the Most Merciful. Without His guidance, mercy, and countless blessings, none of this would have been possible. He has given us the strength, patience, and wisdom to complete this journey, and for that, we are forever thankful.

We would like to extend our sincere thanks to **Dr. Becheke Imène**, our academic supervisor, for her constant support, valuable guidance, and insightful feedback throughout the course of this work. Her encouragement and expertise have played a vital role in the progress and completion of this research.

Our deepest appreciation goes as well to the **examining committee**, particularly **Dr. Bendjama**, who accepted to evaluate our work, and **Dr. Gueddah**, who honored us by presiding over the defense. Their remarks and observations have been of great value to us.

We are also truly grateful to the **engineers and staff of the Laboratory of the University of 20 August 1955, Skikda**, for their assistance, technical support, and kind collaboration. Their welcoming spirit and availability have greatly contributed to the practical part of this project.

To our **families**, especially our **beloved parents**, we owe endless thanks. Their unconditional love, prayers, and sacrifices have been our backbone throughout all the challenges we have faced. Your belief in us means the world.

We also extend our warmest thanks to the **families of our colleagues**, and to all our **friends**, who have encouraged and supported us with kindness, patience, and joy along the way.

Finally, we would like to express our heartfelt gratitude to each other, as a united group (**Haitem, Meroua, Hana and Asma**). Together (four determined and passionate students) we shared the workload, supported one another, and stayed committed through every step of this journey. This collaboration not only made the project a success but also created strong bonds of friendship, respect, and unforgettable memories. We are truly proud of what we have accomplished as a team.

To all of you: **thank you** from the bottom of our hearts.

Dedication:

To my **family**, for their unwavering love and support throughout my academic journey. **My parents** and **siblings** have been my pillars of strength.

I dedicate this work to all who have inspired and motivated me to chase my dreams - my colleagues, friends, and mentors.

To my loved ones, who endured the long hours and hard work that went into this research with patience and understanding. I'm grateful to everyone who contributed to the success of this project.

Special thanks to my friends and colleagues, who stood by me through thick and thin. Your camaraderie and support mean the world to me.

Lastly, to **my parents**, whose selflessness and encouragement have made all the difference.

And to everyone who lent a helping hand, be it financially or emotionally,

I extend my heartfelt gratitude.

L. Haitem

Dedication

This accomplishment, a culmination of years of hard work and dedication, is first and foremost for my beloved parents, Dad and Mum. Your unwavering love, endless sacrifices, and profound belief in my potential have been the bedrock of my life. Without your constant support and encouragement, this journey would have been impossible, and I wouldn't be standing here today.

Thank you for everything.

To my cherished siblings, Youcef, Kenza, and Seif, your presence has always been a source of strength and comfort. And especially to Hana, your unique support and understanding have truly made a difference. Thank you for being my constant cheerleaders.

To my dear friends, who transformed challenging university years into periods filled with laughter, joy, and shared memories, I also extend my heartfelt gratitude to my Scientific Vision Club family; your camaraderie and shared passion provided an invaluable community.

Finally, to my exceptional mentor, Dr. Bechekarr Iman, your insightful guidance, tireless support, and unwavering belief in my abilities have not only shaped this thesis but have also profoundly influenced my academic path. I am deeply grateful for your mentorship.

Meroua

Dedication:

First and foremost, I would like to thank ALLAH without his blessings this work would never see light. To my beloved parents, Ahmed and Zahra, for their unwavering love and support since day one. Your belief in me has been my greatest strength. To my dear siblings, Tina and Fadi, thank you for your constant encouragement and for always being there. To my paternal aunt, Warda, who has encouraged my studies since I was five years old, thank you for your early and continuous support. To my aunt, Radia, for her invaluable assistance in securing my practical internship, your efforts are deeply appreciated. To my aunt, Fouzia, for your constant motivation and belief in my abilities. To my little sister, Rouja, and my little brothers, Idris and Haitem, you bring joy and inspiration to my life. To my cherished grandmother, Yemma Fetouh, for your constant prayers, which have always been a source of comfort and strength. A special thank you to my dear cousin Lamia, my aunt Salima, for your prayers during your Umrah also for your encouragement. Thank you so much. And to my dear friends, Nada, Asma, Jiji, Khaoula, and Sara, and my loving cousins, Camille, Lamis, and Many, your friendship and support mean the world to me. I love you all and thank you for believing in me

Hana

Dedication

"In the name of Allah, the Most Gracious, the Most Merciful."

First and foremost, all praise and gratitude belong to Allah (SWT), by whose infinite blessings and grace I have reached this milestone. Were it not for His favor and mercy, I would not have succeeded.

To my father (may Allah have mercy on him) :

Your advice and words have never left me, I pray that you are pleased and proud of me.

To my mother:

If all the languages of the world were combined, they would still fall short in expressing my gratitude for your sacrifices—your exhaustion, sleepless nights, patience, and unwavering love. Simply put, without you, my dear mother, I would not have succeeded. May Allah reward you with the highest ranks of Paradise. I hope you are proud of your little girl.

To my sisters (Hadjar, Sabah and my love Rayane):

Just having you as my sisters is enough blessing in this life ;thank you for every moment of love, support, and encouragement in my life.

To my brothers ((Abu Bakr / Bob), Mouhamed, Youcef, Soufian and my fiance toufik(titou)):

You are my pillars of strength. Thank you for always standing by me, cheering me on, and being my unwavering support. I pray you are proud of your little sister today and forever.

To (my best friend and soumia):

Thank you for every word of encouragement, every prayer, and every moment of comfort. May we always remain sisters in faith and love.

To (Maram, Salma, Ahmed, Abdeldjalil, Nidal, Ziad, Abdelrahim, and Ayoub):

My beloved nieces and nephews, the joy of my heart—may Allah elevate you to the highest ranks and grant you success in this life and the next.

Finally, to everyone who believed in me, supported me, and prayed for me—thank you from the depths of my heart. I love you all dearly.

—Asma

Table of contents

Abstracts

List of abbreviations

List of figures

List of tables

INTRODUCTION	01
CHAPTER 01 : RESPIRATORY INFECTIONS AND THE ROLE OF HOOKAH-RELATED MICROORGANISMS.....	03
1. Respiratory System :.....	03
1.1. Definition :.....	03
2. Anatomy of the Respiratory System	04
2.1. Upper Respiratory Tract (URT).....	04
2.2. Lower Respiratory Tract (LRT).....	04
3 Respiratory infection.....	05
3.1 Definition.....	05
3.2. Common Pathogens in Respiratory Infections.....	05
3.3. Modes of Transmission.....	06
3.4. Risk Factors	06
4. Microbial Contamination in Hookah.....	07
4.1. Sources of Microorganisms in Hookah Devices.....	07
Chapter 02 :Hookah, electronic cigarettes and health risks.....	08
1. Traditional hookah.....	08
2. Comparison of Hookah, Cigarettes, and Vaping.....	08
2.1. Hookah (Traditional hookah).....	08
2.2. Vaping (Electronic Cigarettes).....	09
2.3. Cigarettes.....	10
3. Infections Transmitted Through Hookah Use.....	10
3.1. Respiratory Infections.....	10
3.1.1. Bacterial Contamination.....	10
3.1.2. Fungal Infections.....	11
3.1.3. Viral Infections.....	11
a. Hepatitis C and other Bloodborne Viruses.....	11

b. Herpes Simplex Virus (HSV-1).....	11
c. Influenza and Common Cold Viruses.....	12
d. COVID-19 and other Coronaviruses.....	12
e. Epstein-Barr Virus (EBV).....	12
3.2. Long-Term Consequences.....	13
3.2.1. Chronic Respiratory Diseases.....	13
4. Preventive Measures.....	13
a. Hygiene Practices.....	13
b. Equipment Maintenance.....	13
c. Personal Precautions.....	14
Material and methods.....	15
1. Material.....	15
1.1. Origins of the samples.....	15
1.2. Sampling protocol for bacterial isolation from <i>shisha</i>	16
1.2.1. Internal sampling (swab).....	16
1.2.2. External Sampling (Swab).....	16
2.3. Filter Sampling.....	17
2. Methods.....	18
2.1. Protocol for Inoculation and Incubation of Samples.....	18
2.1.1. Inoculation Protocol.....	18
a. Sample Preparation.....	18
b. Inoculation Process.....	18
c. Incubation conditions.....	19
d. Identification and Interpretation.....	19
Results.....	21
1. Microorganisms isolated from traditional hookah.....	22
1.1. Bacteriel strains.....	22
2.2. Fungal strains.....	23
2. Microorganisms in electronic cigarettes.....	26
Discussion.....	28
Conclusion.....	30
Recommandations.....	31
<u>Bibliographical references.....</u>	<u>33</u>

Abstract:

The use of hookah (shisha) and electronic cigarettes (e-cigarettes) has witnessed significant growth worldwide, with an alarming increase in prevalence in the Arab world. Hookah smoking, in particular, has deep cultural roots in the Middle East, North Africa, and parts of Asia, while e-cigarettes, marketed as a safer alternative to conventional smoking, have rapidly gained popularity across these regions. Despite widespread perceptions of these alternatives as less harmful than traditional cigarettes, both pose distinct public health risks, particularly concerning infectious diseases and microbial contamination.

The aim of our study was to isolate, identify, and highlight the various microorganisms that can be encountered following the use of traditional shisha or electronic cigarettes, and thus gain a clear understanding of the infectious risk we face. To achieve this, several culture media (standard and selective) were used to isolate as many strains as possible from each sample. Identification was performed using chromagar orientation and API gallery to identify bacteria. For fungi, we used both macroscopic and microscopic identification.

From a microbiological standpoint, both hookah and e-cigarettes present unique risks, primarily related to the potential transmission of harmful microorganisms. Our study showed that the used hookah smoking are not sterile environments. The shared use of the mouthpiece increases the risk of cross-contamination, especially with bacterial and viral pathogens. An important number of pathogen bacteria was isolated from traditional and E-ciragettes in our stady : *S. aureus*, *S. epidermidis*, *E. coli*, *K. pneumoniae*, *Citrobacter sp.*, *Enterobacter sp.*, *Serratia marcescens*, *Streprococcus agalactiae*, *Proteus sp.*, *Salmonella sp.*.

Several fungi were also isolated such as, *Candida albicans*, *Rhodotorula sp.*, *Aspergillus niger*, *Penicillium sp.*, *Fusarium sp.*, *Cladosporium sp.*, *Alternaria sp.*, *Trichoderma sp.*

These various bacterial and fungal species are known to cause different types of infections, including respiratory tract infections, oral lesions, and candidiasis, with varying degrees of severity. In this context, the use of a filter is essential to prevent cross-contamination between users, thereby reducing the risk of microorganism transmission.

In our study, only one tea lounge provides filters to its customers. This lack of precaution in other establishments reflects an irresponsible attitude, exposing consumers to a significant public health risk especially in light of the growing spread of antibiotic-resistant strains.

Key words : Bacteria, E-cigarettes, Filter, Fungi, Hookah, Respiratory infections

Résumé

L'utilisation de la chicha (narguilé) et des cigarettes électroniques connaît une croissance significative à travers le monde, avec une augmentation particulièrement alarmante dans le monde arabe. La chicha, en particulier, possède des racines culturelles profondes au Moyen-Orient, en Afrique du Nord et dans certaines régions d'Asie, tandis que les cigarettes électroniques, commercialisées comme une alternative plus sûre au tabac traditionnel, ont rapidement gagné en popularité dans ces régions. Malgré une perception largement répandue selon laquelle ces alternatives seraient moins nocives que les cigarettes classiques, elles présentent toutes deux des risques sanitaires distincts, notamment en matière de maladies infectieuses et de contamination microbienne.

Notre étude avait pour objectif d'isoler, d'identifier et de mettre en évidence les différents microorganismes pouvant être rencontrés suite à l'utilisation de chicha traditionnelle ou de cigarettes électroniques, afin de mieux comprendre les risques infectieux encourus. Pour cela, plusieurs milieux de culture (standards et sélectifs) ont été utilisés afin d'isoler un maximum de souches à partir de chaque échantillon. L'identification bactérienne a été réalisée à l'aide du Chromagar Orientation et de la galerie API. Pour les champignons, une identification macroscopique et microscopique a été effectuée.

D'un point de vue microbiologique, la chicha et les cigarettes électroniques présentent des risques spécifiques, liés principalement à la transmission potentielle de microorganismes pathogènes. Notre étude a montré que les dispositifs de chicha ne sont pas des environnements stériles. Le partage de l'embout buccal augmente le risque de contamination croisée, en particulier par des agents pathogènes bactériens et viraux. Un nombre important de bactéries pathogènes a été isolé dans notre étude : *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter sp.*, *Enterobacter sp.*, *Serratia marcescens*, *Streptococcus agalactiae*, *Proteus sp.*, *Salmonella sp.*

Plusieurs champignons ont également été isolés, tels que *Candida albicans*, *Rhodotorula sp.*, *Aspergillus niger*, *Penicillium sp.*, *Fusarium sp.*, *Cladosporium sp.*, *Alternaria sp.*, *Trichoderma sp.*

Ces différentes espèces bactériennes et fongiques sont connues pour être responsables de divers types d'infections, notamment des infections respiratoires, des lésions buccales et des candidoses, avec des degrés de gravité variables.

Dans ce contexte, l'utilisation d'un filtre est essentielle pour éviter la contamination croisée entre les utilisateurs et réduire le risque de transmission des microorganismes.

Dans notre étude, seul un salon de thé fournissait des filtres à ses clients. Le manque de précaution dans les autres établissements reflète une attitude irresponsable, exposant les consommateurs à un risque sanitaire important, notamment face à la propagation croissante des souches résistantes aux antibiotiques.

Mots-clés : Bactéries, Cigarettes électroniques, Filtre, Champignons, Chicha, Infections respiratoires

الملخص

شهد استخدام الشيشة والسجائر الإلكترونية نموًا ملحوظًا في جميع أنحاء العالم، مع تزايد مثير للقلق في العالم العربي. فالشيشة لها جذور ثقافية عميقة في الشرق الأوسط وشمال إفريقيا وأجزاء من آسيا، بينما تُسوّق السجائر الإلكترونية كبديل أكثر أمانًا للتدخين التقليدي، وقد أصبحت شائعة بسرعة في هذه المناطق. على الرغم من الاعتقاد السائد بأن هذه البدائل أقل ضررًا من السجائر التقليدية، إلا أنها تُشكّل مخاطر صحية عامة مميزة، خاصةً فيما يتعلق بالأمراض المعدية والتلوث الميكروبي.

كان هدف دراستنا هو عزل وتحديد وتسليط الضوء على الكائنات الدقيقة المختلفة التي قد توجد بعد استخدام الشيشة التقليدية أو السجائر الإلكترونية، وذلك لفهم واضح للمخاطر العدوى التي نواجهها. لتحقيق ذلك، تم استخدام عدة أنواع من الأوساط الزرعية (العادية والانتقائية) لعزل أكبر عدد ممكن من السلالات من كل عينة. تم التعرف على البكتيريا باستخدام وسط الكروم آغار ومجموعة API ، أما الفطريات فقد تم التعرف عليها بواسطة الفحص المجهرى والعيانى.

من منظور ميكروبيولوجي، تمثل الشيشة والسجائر الإلكترونية مخاطر مميزة، تتعلق أساسًا بإمكانية نقل الكائنات الدقيقة الضارة. وقد أظهرت دراستنا أن أدوات الشيشة ليست بيئات معقمة. فمشاركة فم الشيشة بين المستخدمين ترفع من خطر العدوى المتبادلة، خصوصًا من مسببات الأمراض البكتيرية والفيروسية. وقد عزلنا في دراستنا عددًا كبيرًا من البكتيريا الممرضة مثل:

Staphylococcus aureus, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*,
Citrobacter sp., *Enterobacter sp.*, *Serratia marcescens*, *Streptococcus agalactiae*, *Proteus sp.*,
Salmonella sp.

كما تم عزل عدة أنواع من الفطريات مثل *Candida albicans*, *Rhodotorula sp.*, *Aspergillus niger*, *Penicillium sp.*, *Fusarium sp.*, *Cladosporium sp.*, *Alternaria sp.*, *Trichoderma sp.*

تُعرف هذه الأنواع من البكتيريا والفطريات بتسببها في أنواع متعددة من العدوى، مثل التهابات الجهاز التنفسي، والآفات الفموية، وداء المبيضات، بدرجات خطورة متفاوتة.

وفي هذا السياق، فإن استخدام الفلتر يعد ضروريًا لمنع انتقال العدوى بين المستخدمين، وبالتالي تقليل خطر انتقال الكائنات الدقيقة.

وفي دراستنا، كان هناك صالون شاي واحد فقط يوفر الفلاتر لزبائنه. يعكس غياب هذه الإجراءات في بقية الأماكن سلوكًا غير

مسؤول، مما يعرض المستهلكين لخطر صحي كبير، خاصة في ظل الانتشار المتزايد للسلاسل المقاومة للمضادات الحيوية.

الكلمات المفتاحية: البكتيريا، السجائر الإلكترونية، الفلتر، الفطريات، الشيشة، الالتهابات التنفسية

List of Abbreviations

WTS : waterpipe tobacco smoking

LRT :_Lower Respiratory Tract

URT :_Upper Respiratory Tract

TB : Tuberculosis

HIV/AIDS : Human Immunodeficiency Virus / Acquired Immunodeficiency Syndrome

COPD : **Chronic Obstructive Pulmonary Disease**

CNS : Coagulase-negative staphylococci

MRSA : Methicillin-Resistant *Staphylococcus aureus*

UTIs : Urinary Tract Infections

PAHs : Polycyclic Aromatic Hydrocarbons

PG : propylene glycol

VG : vegetable glycerin

Number	Title	Page
01	Respiratory System	04
02	Traditional hookah	09
03	Vaping (Electronic Cigarettes)	09
04	Cigarettes	10
05	internal sampling	16
06	External Sampling	17
07	Hookahs sampling	17
08	swab inoculation method	19
09	fresh state (fungi)	20
10	Gram straining	20
11	Results of seeding different samples of hookah and E-cigarette	21

Liste of figures

List of tables :

Number	Title	Page
<u>01</u>	Common Respiratory Pathogens and Their Characteristics	06
<u>02</u>	Samples of traditional hookah	15
<u>03</u>	Bacterial strains isolated from traditional hookah.	22
<u>04</u>	Fungi strains isolated from traditional hookah.	24
<u>05</u>	microorganisms in e-cigarettes	27

A decorative scroll graphic with the word "INTRODUCTION" written on it. The scroll is horizontal and has a slight curve at the ends, with small circular details at the top corners suggesting it is unrolled. The text is in a bold, black, serif font.

INTRODUCTION

The hookah, also known as shisha or waterpipe, has a rich and complex history that spans over 500 years. Originating in India and Persia (modern-day Iran) in the 16th century, the hookah was initially used by nobility and intellectuals as a symbol of wealth and status.

The device was designed to filter tobacco smoke through water, which was believed to purify the smoke and make it less harsh on the lungs. The hookah quickly gained popularity among the upper classes, and its use spread throughout the Middle East, North Africa, and Asia.

Over time, the hookah became an integral part of social gatherings and cultural events, particularly in countries such as Egypt, Turkey, and Morocco. The device was often decorated with intricate designs and ornaments, reflecting the craftsmanship and artistry of the region (**Maziak et al., 2025**).

Tobacco consumption continues to take many forms worldwide, with hookahs and electronic cigarettes (e-cigarettes) representing two widely used alternatives to conventional smoking. While often perceived as safer, both devices have been implicated in the transmission of bacterial pathogens hookahs through shared mouthpieces and contaminated water pipes, and e-cigarettes through contaminated e-liquids and impaired host defenses (**Martinasek et al., 2018**).

Hookah-associated bacterial transmission arises mainly from the communal use of hoses and mouthpieces. Microbiological surveys of hookah equipment have revealed a diverse array of bacteria, including *Mycobacterium tuberculosis*, *Helicobacter pylori*, *Aspergillus spp.*, and antibiotic-resistant strains of *Staphylococcus* and *Pseudomonas* localized in the apparatus and tobacco itself (**Martinasek et al., 2018; Akl et al., 2010**). For instance, outbreaks of tuberculosis have been traced to shared hookah use, confirming waterpipe smoking as a viable route for infection transmission (**Munckhof et al., 2003**).

Moreover, emerging metagenomic analyses highlight that hookah tobaccos harbor opportunistic pathogens such as *Bacillus cereus* and *Propionibacterium acnes*, both implicated in respiratory and pleural infections (Sapkota et al., 2022). These findings support concerns that waterpipe use may facilitate bacterial transmission via shared equipment and contaminated tobacco substrates (**Sapkota et al., 2022**).

Although e-cigarettes do not involve combustion, they present distinct microbiological hazards. A study analyzing 75 popular e-cigarette products found endotoxins (27%) and fungal glucans (81%), identified in both flavorings and cartridge materials, typically originating from contaminated cotton wicks or e-liquid components (Harvard T.H. Chan School of Public Health, 2019). Immunological investigations reveal that e-cigarette aerosols can suppress innate immune functions: neutrophils

exhibit impaired chemotaxis (4.2-fold), reduced ROS production (48%), and decreased NET formation, leading to diminished clearance of bacteria like *Pseudomonas aeruginosa* and increased bacterial burden in lungs in animal models (**Corriden et al., 2019**). Similarly, respiratory epithelial cells exposed to vapor show reduced antimicrobial peptide production, facilitating biofilm formation and virulence in pathogens like *Staphylococcus aureus* (**Pushalkar et al., 2022**). These immunomodulatory effects make e-cigarette users more susceptible to respiratory and systemic bacterial infections.

E-cigarette aerosol inhalation significantly alters the oral microbiota, shifting it toward a more pathogenic profile. Users exhibit increased dental plaque, elevated colonization by *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, and heightened presence of *S. aureus*, associated with periodontitis (**Gilpin et al., 2019**).



**CHAPTER 01 : RESPIRATORY INFECTIONS AND THE ROLE OF
HOOKAH-RELATED MICROORGANISMS**

Respiratory infections remain a major global health burden, contributing to significant morbidity and mortality across diverse populations. These infections range from mild illnesses, such as the common cold, to severe conditions like pneumonia and tuberculosis. Risk factors include environmental exposures, immunological status, and behavioral practices such as smoking (U.S. Department of Health and Human Services, 2014). Among smoking-related behaviors, waterpipe tobacco smoking (WTS) use has gained popularity, particularly among young adults, who often perceive it as less harmful and addictive compared to traditional cigarette smoking, a misconception reinforced by social norms and the lack of immediate negative health experiences (Salles and *al.*, 2023)."

, However, emerging evidence suggests that hookah smoking not only exacerbates respiratory infections by impairing lung function and immune defenses but also facilitates microbial transmission due to its communal nature and inadequate hygiene practices (Akl et *al.*, 2010; Bhatnagar et *al.*, 2019).

Hookah smoking involves inhaling flavored tobacco smoke filtered through a water basin, a practice historically rooted in Middle Eastern and South Asian cultures (Patel et *al.*, 2019). Despite its social appeal, hookah smoke contains harmful substances, including nicotine, carbon monoxide, heavy metals, and carcinogenic compounds like polycyclic aromatic hydrocarbons, which damage the respiratory epithelium and increase infection susceptibility (Shihadeh et *al.*, 2015; El-Zaatari et *al.*, 2015). Additionally, *The warm and humid conditions inside waterpipe hoses create an ideal environment for microbial biofilm development, facilitating the persistence and spread of pathogenic bacteria and fungi. This biofilm formation not only enhances the survival of harmful microorganisms but also increases the risk of their transmission to users, contributing to oral dysbiosis and potential systemic health complications (Senaratne et al., 2023).*" Shared mouthpieces further facilitate the spread of respiratory viruses, including influenza and SARS-CoV-2, particularly in social smoking settings (World Health Organization [WHO], 2021).

1. Respiratory System :

1.1. Definition :

The respiratory system is a biological system responsible for gas exchange, enabling the intake of oxygen (O₂) and the expulsion of carbon dioxide (CO₂). It ensures the delivery of oxygen to tissues and the removal of metabolic waste, which is critical for cellular respiration and homeostasis (López et Martinson, 2017).

2. Anatomy of the Respiratory System :

The respiratory system consists of the upper and lower respiratory tracts (**López & Martinson, 2017**) (**Figure 01**) :

2.1. Upper Respiratory Tract (URT) :

- ❖ **Nose and Nasal Cavity:** Filters, warms, and humidifies inhaled air.
- ❖ **Pharynx:** A shared pathway for air and food.
- ❖ **Larynx:** Contains vocal cords and prevents food entry into the trachea.

2.2. Lower Respiratory Tract (LRT) :

- ❖ **Trachea:** A cartilaginous tube conducting air to the bronchi.
- ❖ **Bronchi and Bronchioles:** Branching airways that distribute air to the lungs.
- ❖ **Lungs:** Paired organs where gas exchange occurs in the alveoli.
- ❖ **Alveoli:** Tiny sacs surrounded by capillaries, facilitating O₂ and CO₂ exchange.

The system also includes accessory structures like the **pleurae** (protective membranes around the lungs) and the **diaphragm** (a muscle aiding inhalation and exhalation).

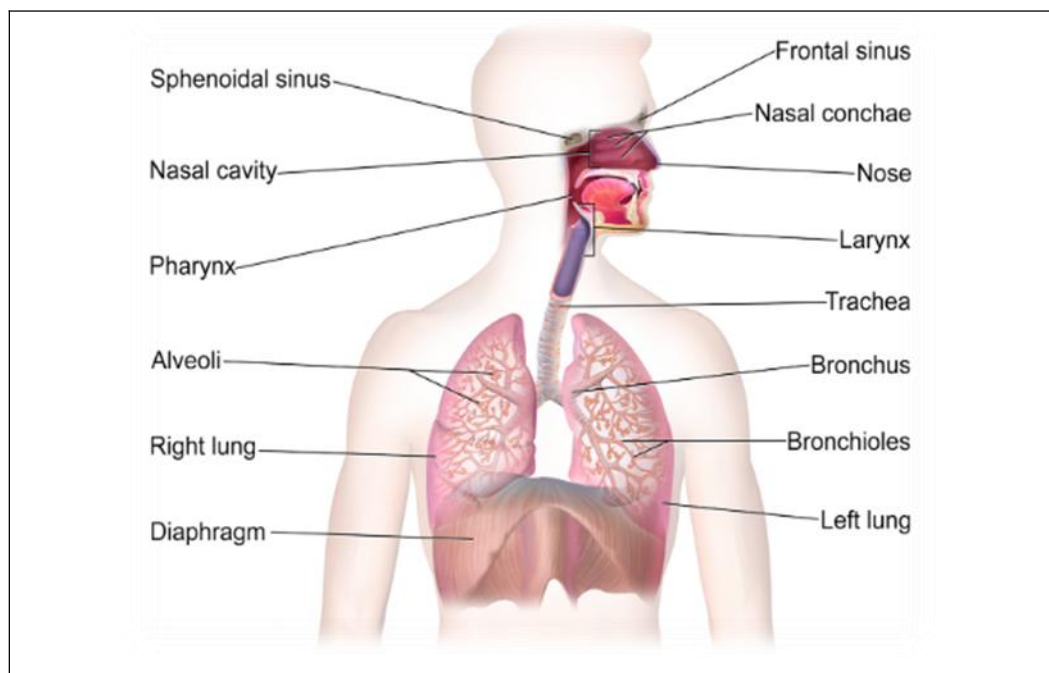


Figure 01: Respiratory System

3 Respiratory infection :

3.1 Definition :

Respiratory infections are diseases affecting the LRT or URT, caused by pathogenic microorganisms such as bacteria, viruses, or fungi. These infections can range from mild illnesses like the common cold to severe conditions such as pneumonia and tuberculosis. The respiratory system's exposure to environmental pollutants, including tobacco smoke, increases susceptibility to infections by compromising immune defenses and damaging mucosal barriers (Bahtouee et al., 2017).

3.2. Common Pathogens in Respiratory Infections :

Respiratory infections are caused by various pathogens, including bacteria, viruses, and fungi. Some of the most common microorganisms associated with respiratory diseases include:

❖ Bacterial Pathogens :

- *Streptococcus pneumoniae*: a leading cause of community-acquired pneumonia (Bahtouee et al., 2017; Weiser et al., 2018).
- *Mycobacterium tuberculosis* : The causative agent of tuberculosis (TB), transmitted via airborne droplets (Chaouachi, 2009; WHO, 2022).
- *Pseudomonas aeruginosa*: An opportunistic pathogen linked to chronic lung infections (Bahtouee et al., 2017 ; Lyczak et al., 2000).

❖ Fungal Pathogens :

- *Aspergillus spp.* : Opportunistic fungi causing aspergillosis, particularly in immunocompromised individuals and hookah users due to contaminated water reservoirs and shared mouthpieces (Patel et al., 2019; Qasim et al., 2019).

❖ Viral Pathogens :

- **Influenza virus** : Causes seasonal epidemics and severe respiratory complications (Bahtouee et al., 2017; Iuliano et al., 2018).
- **Respiratory syncytial virus (RSV)** : Predominantly affects children and immunocompromised individuals (Shi et al., 2017).

3.3. Modes of Transmission :

- **Airborne droplets** : Expelled through coughing or sneezing (e.g., TB, influenza) (Tellier, 2009).
- **Direct contact**: Via contaminated hands or shared hookah mouthpieces (Al-Nour Gassim et al., 2023).
- **Fomites**: Pathogens persist on hookah components (Ancigil et Arbag, 2018).

3.4. Risk Factors (Table 01):

- **Immunocompromised states**: HIV/AIDS, diabetes (Fishman, 2007).
- **Smoking**: Impairs mucociliary clearance (Arcavi et Benowitz, 2004).

Table 1: Common Respiratory Pathogens and Their Characteristics (Patel et al., (2019); Qasim et al.,(2019).

4.

Pathogen	Type	Transmission Route	Associated Diseases	Risk Factors
<u><i>Streptococcus pneumoniae</i></u>	Bacterium	Airborne droplets, direct contact	Pneumonia, sinusitis	Smoking, immunocompromised
<u><i>Mycobacterium tuberculosis</i></u>	Bacterium	Airborne droplets	Tuberculosis (TB)	Crowded environments, smoking
<u><i>Influenza virus</i></u>	virus	Airborne droplets, fomites	Influenza, pneumonia	Seasonal exposure, smoking
<u><i>Pseudomonas aeruginosa</i></u>	Bacterium	Direct contact, fomites	Pneumonia, bronchitis	Chronic lung disease, smoking
<u><i>Aspergillus spp.</i></u>	Fungus	Airborne spores	Aspergillosis	Immunocompromised, hookah use

4. Microbial Contamination in Hookah :

Hookah devices provide multiple niches for microbial colonization due to their complex structure, moist environment, and frequent use without proper sterilization. The microorganisms colonizing these devices are not merely commensals; many are opportunistic pathogens capable of causing respiratory and systemic infections (Altindis et al., 2020).

4.1. Sources of Microorganisms in Hookah Devices :

- ❖ Mouthpieces and Hoses : the **interior and exterior surfaces** of fixed mouthpieces and hoses are heavily contaminated with bacteria from users' oral flora and hands.

Common isolates:

- *Staphylococcus aureus* (including MRSA), coagulase-negative staphylococci (CNS), *Streptococcus spp.*, *Enterococcus spp.* (Altindis et al., 2020; Shakhathreh et al., 2018).
 - *Staphylococcus pasteurii*, *Bacillus cereus*, *Microbacterium paraoxydans* (Martinasek et al., 2018).
- **Contamination rates:**
 - **69–100%** in mouthpieces (Safizadeh et al., 2014; Martinasek et al., 2018).
 - **57.7–74.2%** in hoses (Altindis et al., 2020).

- ❖ **Water Bowls**

The **water reservoir**, often unchanged between sessions, harbors **Gram-negative bacteria** and biofilms.

Common pathogens :

- *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* (Altindis et al., 2020; Aljadani et al., 2020).
- *Enterobacter cloacae*, *Flavobacterium* spp. (Alaidarous et al., 2017).

Contamination rates: 23.1–86% (Altindis et al., 2020 ; Alaidarous et al., 2017).

- ❖ **Tobacco and Charcoal :**

- Flavored tobacco mixtures and charcoal may introduce **fungus contaminants** (*Candida albicans*) and bacteria (Shakhathreh et al., 2018).
- Moist tobacco (*maassel*) supports microbial growth (Alqumber, 2021)



Chapter 02 :Hookah, electronic cigarettes and health risks

1. Traditional hookah :

Hookah smoking, often perceived as a social and less harmful alternative to cigarettes, poses significant health risks according to the Centers for Disease Control and Prevention (CDC, 2025). Despite the water filtration system, hookah smoke contains numerous toxic substances, including nicotine, carbon monoxide, heavy metals, and carcinogens (Cleveland Clinic, 2024).

A single hookah session can expose users to a volume of smoke equivalent to smoking multiple cigarettes, exacerbating the potential for health complications. To understand the risks, it is essential to distinguish between hookah, vaping and cigarettes (REF).

2. Comparison of Hookah, Cigarettes, and Vaping:

2.1. Hookah (Traditional hookah) :

- Composition: Flavored tobacco (shisha) mixed with molasses, honey, or fruit pulp.
- Mechanism: Hot coals heat the shisha, producing smoke that passes through water before inhalation (**Figure 1**).
- Health Risks: High exposure to toxins, including nicotine, carbon monoxide, and carcinogens. An hour of hookah smoking can equal 100 cigarettes (Cleveland Clinic, 2024).

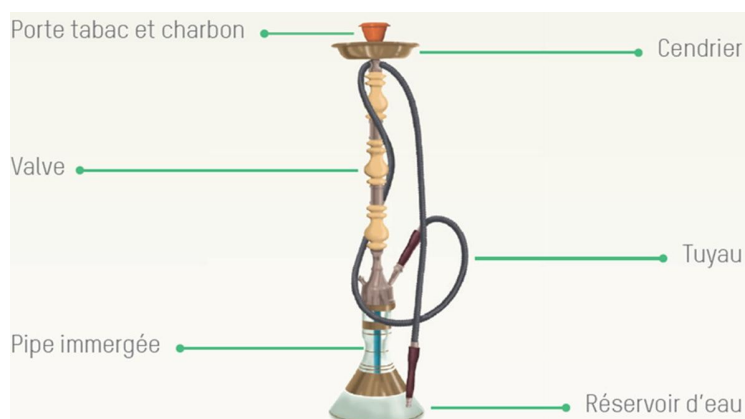


Figure 02: Traditional hookah

2.2. Vaping (Electronic Cigarettes):

- **Composition:** Liquids containing nicotine, flavorings, and other chemicals.
- **Mechanism:** Heating the liquid to create an aerosol for inhalation (**Figure 2**).
- **Health Risks:** Lung injuries, nicotine addiction, and environmental pollution from device waste (Cleveland Clinic, 2024).



Figure 03: Vaping (Electronic Cigarettes)

2.3. Cigarettes:

- **Composition** : Rolled paper filled with tobacco and additives (**Figure 3**).
- **Mechanism** : Burning tobacco to produce smoke for inhalation.
- **Health Risks** : Lung cancer, heart disease, and respiratory problems due to thousands of chemicals, including tar and carbon monoxide (Cleveland Clinic, 2024).



Figure 04: Cigarettes

3. Infections Transmitted Through Hookah Use:

Hookah use, especially when shared, significantly increases the risk of various infections due to the moist environment and communal sharing of mouthpieces (REF).

3.1. Respiratory Infections :

3.1.1. Bacterial Contamination :

Hookah tobacco can harbor bacteria like *Pseudomonas aeruginosa*, *Klebsiella* species, *Escherichia coli*, and *Enterobacter* species (**Mohamed et al., 2022**).

These bacteria thrive in the moist environment of the hookah and can be inhaled, leading to respiratory infections (Université du Maryland, 2024).

Examples :

- ✓ A person with a mild bacterial infection can contaminate the hookah water, which is then inhaled by subsequent users, leading to pneumonia or bronchitis (REF).
- ✓ Outbreaks of pulmonary tuberculosis have been linked to shared waterpipe use. (WHO FCTC, n.d.)

3.1.2. Fungal Infections:

Fungi species like *Candida*, *Fusarium*, and *Aspergillus* can contaminate hookah components. These can cause respiratory issues, especially in individuals with weakened immune systems (Alharhi et al., 2018).

Example:

Inhaling spores of *Aspergillus* can lead to aspergillosis, a lung infection, particularly dangerous for those with asthma or chronic lung disease (Centers for Disease Control and Prevention, n.d.).

3.1.3. Viral Infections:**a. Hepatitis C and other Bloodborne Viruses:**

Sharing mouthpieces can transmit bloodborne viruses like hepatitis C through contaminated saliva or blood from microabrasions in the mouth (WHO, n.d.)

Example:

If a person with hepatitis C has bleeding gums and shares a hookah, the virus can be transmitted to other users.

b. Herpes Simplex Virus (HSV-1):

HSV-1, causing oral herpes, spreads through saliva on shared mouthpieces (CDC, 2023)

Example:

Sharing a hookah with someone who has an active oral herpes outbreak significantly increases the risk of transmission.

c. Influenza and Common Cold Viruses:

Respiratory viruses like influenza and rhinoviruses survive on moist surfaces and spread through shared hookah pipes (American Lung Association, 2022).

Example:

During flu season, sharing a hookah can lead to rapid transmission of the influenza virus among users.

d. COVID-19 and other Coronaviruses:

The close proximity and shared equipment in hookah use increase the risk of COVID-19 transmission. SARS-CoV-2 can persist on moist surfaces and aerosolize during smoking (WHO FCTC, 2020).

Example:

An individual with an asymptomatic COVID-19 infection using a shared hookah can spread the virus to others.

e. Epstein-Barr Virus (EBV):

EBV, responsible for mononucleosis, spreads through saliva, making shared hookah use a high-risk activity (Johns Hopkins Medicine, 2021).

Example:

A young adult sharing a hookah at a social gathering can contract EBV from an infected individual, leading to mono.

3.2. Long-Term Consequences :

3.2.1. Chronic Respiratory Diseases: Frequent hookah use can lead to chronic bronchitis, emphysema, and increased susceptibility to pulmonary infections.

- **Increased risk of Tuberculosis:** Sharing hookahs increases the spread of Tuberculosis.
- **Cardiovascular Diseases:** The toxic chemicals in hookah smoke can damage the cardiovascular system, increasing the risk of heart disease and stroke.
- **Cancer:** Hookah smoke contains carcinogens, raising the risk of lung, oral, and other cancers (Mayo Clinic, n.d.).
- **Compromised Immune System:** Long term use can weaken the immune system, leading to an

increased likelihood of all types of infections (Cleveland Clinic,n.d.).

4. Preventive Measures :

a. Hygiene Practices :

- Thoroughly clean and disinfect hookah equipment after each use.
- Use disposable mouthpieces and avoid sharing.
- Wash hands before and after handling hookah equipment.

b. Equipment Maintenance :

- Regularly change the water in the hookah to minimize bacterial and fungal growth.
- Ensure proper ventilation in smoking areas.

c. Personal Precautions:

- Avoid using hookahs in crowded or poorly ventilated areas.
- Individuals with respiratory conditions or weakened immune systems should avoid hookah use altogether.
- Educate users on the risks of sharing hookahs.

The practice of hookah smoking, often perceived as a social and relatively benign activity, presents a significant risk for the transmission of a wide array of infections. The unique characteristics of hookah use, including the moist environment of the water pipe and the frequent sharing of mouthpieces, create an ideal setting for the proliferation and transmission of bacterial, fungal, and viral pathogens.

The evidence presented highlights the potential for serious respiratory infections, bloodborne viral infections, and other communicable diseases associated with hookah use.

Beyond the immediate risk of infection, the long-term health consequences of regular hookah consumption are substantial. The chronic exposure to toxins and pathogens can lead to severe respiratory diseases, cardiovascular complications, and an increased risk of cancer. Therefore, it is imperative that public health messaging emphasizes the potential dangers of hookah use, particularly in social settings where sharing is common.



Material and methods

Shisha and e-cigarette use has become very common and is a fad, especially among young people. However, this phenomenon poses a serious public health problem, not only linked to the harmful effects and toxicity of nicotine, but also to infectious diseases that can be transmitted through the use of hookah or e-cigarettes, which are often shared among young people.

The goal of our work is to isolate, identify, and detect microbial strains that can colonize shisha, especially the mouthpiece (or sleeve) of the hose, often used without a filter, the filters, and the mouthpiece of e-cigarettes. These sites are the source of the transmission of these microorganisms and, consequently, the transmission of several diseases such as respiratory infections, candidiasis, and others.

1. Material :

1.1. Origins of the samples:

For this study, we were able to collect surface samples and filters used at two different tea shops (S1 and S2) in the province of Skikda for traditional hookah. Two samples of e-cigarettes were taken from the university (students).

Table 02: Samples of traditional hookah

Traditional hookah	
Tea Shop 1 (S1)	Tea shop 2 (S2)
3 samples (3 different hookahs)	2 samples (2different hookahs)
3 internal samples (mouthpiece)	3 internal samples (mouthpiece)
3 external samples (mouthpiece)	3 external samples (mouthpiece)
2 filters	/

1.2. Sampling protocol for bacterial isolation from *shisha* :

1.2.1. Internal sampling (swab) :

Internal sampling was performed using sterile swabs. A swab was inserted into the inner section of the *shisha* (pipe) to collect a sample directly from areas that could be contaminated by microorganisms. The swab was gently used to avoid disturbing the microbial environment. It was then immediately placed into a sterile transport tube containing an appropriate culture medium to preserve the bacteria until further analysis. Each tube was uniquely labeled to ensure proper sample traceability and prevent any mix-up during subsequent microbiological analysis.(figure01)

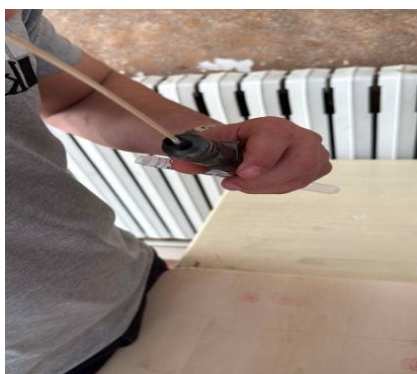


Figure 05 : Internal sampling (Personal take)

1.2.2. External Sampling (Swab) :

External sampling was also carried out using sterile swabs, targeting various external areas of the *shisha*, particularly the mouthpiece. These areas are prone to contamination, especially through direct contact with hands or environmental surfaces, which may act as vectors for bacteria. Each swab was then placed into a sterile transport tube containing a suitable medium for the preservation of the microorganisms. As with internal sampling, each tube was clearly labeled to ensure proper sample management. (figures02and03)

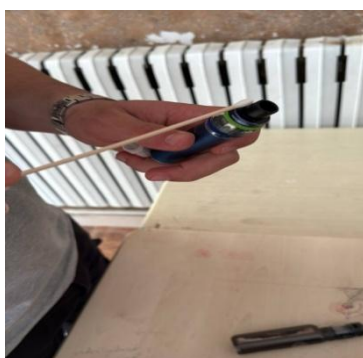


Figure 06: External Sampling



Figure07: Hookahs sampling (Personal take)

2.3. Filter Sampling :

Samples were also collected from the filters of both *shishas*, with one filter taken from each. These filters, often used to filter out impurities or nicotine, can serve as sites for bacterial growth due to the moisture and residue present. A filter was collected from each *shisha* and placed into a sterile transport tube containing an appropriate culture medium. This step helps to detect any potential pathogens that could be trapped in the filters and potentially contaminate the user.

2. Methods:

2.1. Protocol for Inoculation and Incubation of Samples :

2.1.1. Inoculation Protocol :

a. Sample Preparation:

Ensure that all collected samples (swabs or filters) are properly labeled and kept in sterile transport tubes until inoculation.

Four culture medias were used :

- Nutrient agar : for the cultivation of a wide variety of bacteria (growth of non-selective bacterial).
- Chapman Agar (Selective for *Staphylococcus aureus*).
- Hektoen Enteric Agar (Selective for *Enterobacteriaceae*).
- Sabouraud Agar (Selective for Fungi).

b. Inoculation Process :

Adding sterile saline water to each swab to preparing bacterial suspension. Using the swab, transfer a small aliquot of the sample onto the surface of each agar plate. Gently streak the sample across the agar surface using a zigzag motion to allow for proper bacterial growth and isolation .

For filters, the same method was used. (figure 04)

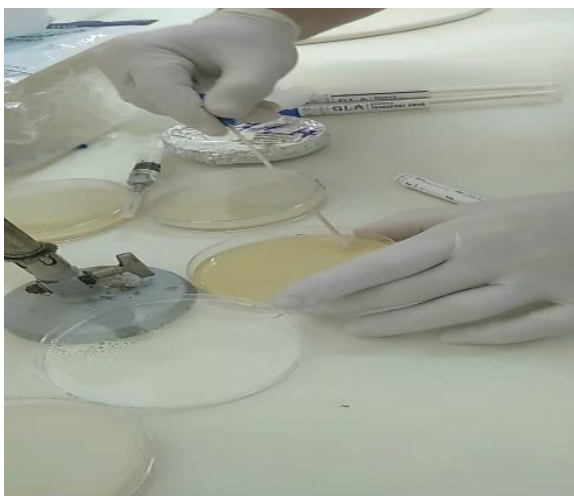


Figure 08 : swab inoculation method (Personal take)

c. Incubation conditions :

- For the bacterial growth :

Incubate Nutrient Agar Hektoen and Chapman Agar plates at 37°C for 18-24 hours for optimal bacterial growth.

- For fungal Growth:

Incubate Sabouraud Agar at 30°C for 3–7 days to allow fungal colonies to develop. Observe for distinct fungal morphology such as filamentous structures or yeast-like colonies.

d. Identification and Interpretation :

After incubation, carefully examine the growth on each medium:

- Bacterial colonies: Check for characteristics like shape, size, color, and hemolysis (for nutrient agar).

- Confirm suspected *S. aureus* colonies on Chapman agar by API system Staph.
- **Fungal colonies:** Observe macroscopic colony features like color, texture, and any signs of mycelial growth (for Sabouraud agar).

Enterobacteriaceae: Confirm suspected colonies from Hektoen agar with Chromagar and biochemical tests (API System).

Identification of idiol species differences was preceded by a fresh state plus Gram staining .(figures 05 and06)

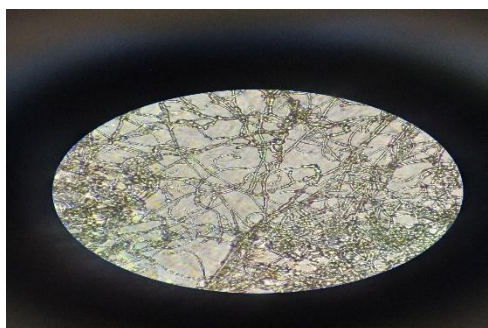


Figure 09 : Fresh state(fungi)(**Personal take**)

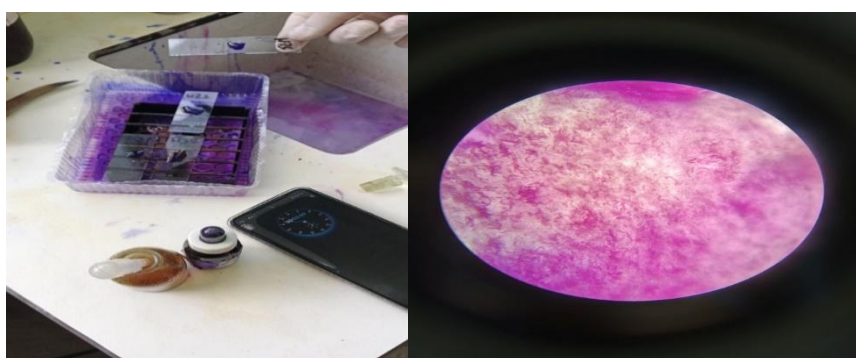


Figure 10 : Gram straining (**Personal take**).



Results

After incubation, a very high number of bacteria and fungi were noted on the various containers for all samples taken (Figure ..). This indicates the high contamination of the hookah mouthpiece as well as the e-cigarette. And this result raises the alarm about the risk encountered following the possible cross-contamination between consumers.

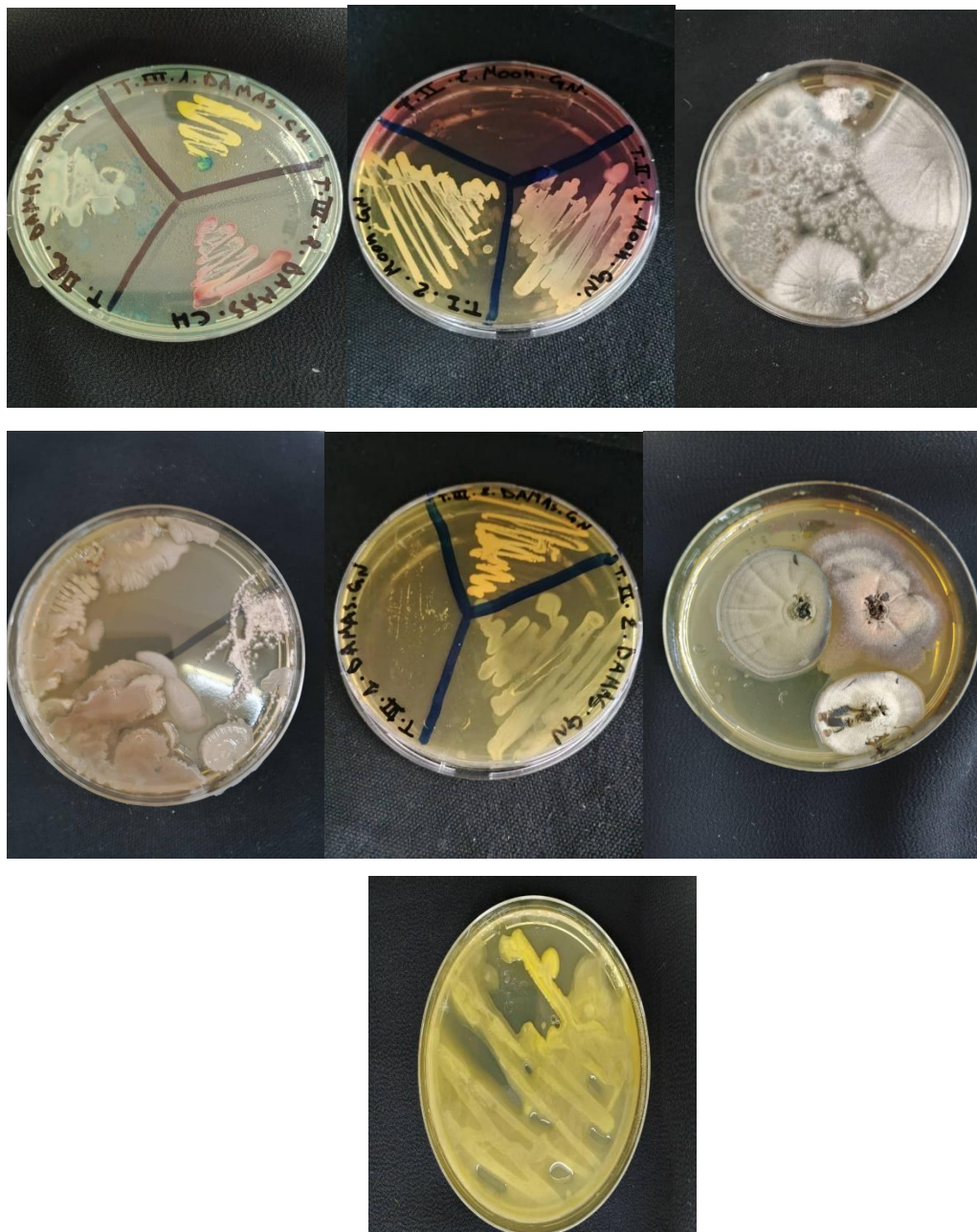


Figure 11 : Results of seeding different samples of hookah and E-cigarette
(Personal take).

1. Microorganisms isolated from traditional hookah :

1.1. Bacteriel strains:

The microbiological analysis of traditional hookah components from two different tea lounges or tea shops (S1 and S2) revealed various bacterial and fungal species, confirming notable microbial contamination.

We note the presence of pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.*, and members of the KESC group (*Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter*) were identified, posing risks of infections, especially in immunocompromised individuals.

The first tea shop (S1) was found to be more contaminated than the second with a very high and more varied number of bacteria (Table 04), (Figure 11).

Candida spp. was also found, known for causing candidiasis and oral ulcers (aphthae).

The presence of microorganisms in the filters highlights their importance in contamination and the need for regular cleaning or replacement to reduce microbial risk.

Table 03 : Bacterial strains isolated from traditional hookah.

	Sample	Internal sampling (1)	External sampling (2)
Tea Shop 1 (S1)	Hookah 1	<i>Staphylococcus aureus</i> , <i>Salmonella spp</i>	<i>Staphylococcus aureus</i> , <i>Salmonella spp</i> , <i>Klebsiella</i> , <i>Enterobacter</i> , <i>Serratia</i> , <i>Citrobacter</i> (KESC)
	Hookah 2	<i>Klebsiella</i> , <i>Enterobacter</i> , <i>Serratia</i>	<i>Staphylococcus aureus</i> , <i>Klebsiella</i> , <i>Enterobacter</i> , <i>Serratia</i>
	Hookah 3	<i>Staphylococcus</i> <i>epidermidis</i> , <i>Klebsiella</i> , <i>Enterobacter</i>	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i>
Tea shop 2 (S2)	Hookah 1	<i>Enterococcus</i> , <i>S. epidermidis</i>	<i>Staphylococcus aureus</i>
	Hookah 2	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>

Filters	
Filter 1 Tea Shop 1 (S1)	<i>Staphylococcus saprophyticus</i> , <i>Klebsiella</i> , <i>Enterobacter</i> , <i>Staphylococcus epidermidis</i>
Filter 2 Tea Shop 1 (S1)	<i>Enterococcus</i> , <i>Staphylococcus epidermidis</i> , <i>Candida</i>

2.2. Fungal strains :





Macroscopic and microscopic identification of fungi obtained on agar Sabouraud showed different fungal genera, including *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Alternaria*, *Trichoderma*, *Rhodotorula*, and *Candida* (**Table 04**).



Some of these fungi are potentially harmful. For example, *Aspergillus niger* can cause lung infections, and *Alternaria* and *Fusarium* are known for producing toxins. *Penicillium* may cause allergies and respiratory problems.



***Candida* spp., seen as small colonies, is responsible for candidiasis and oral ulcers (aphthae).**

This shows that traditional hookahs can be contaminated by fungi that may affect human health.

Table 04 : Fungi strains isolated from traditional hookah.

Petri dishes	Macroscopic Description	Probable Genus	Comments	Photo
1	Two main colonies: one white with central umbo and concentric rings; one brownish with a cottony texture	<i>Penicillium</i> or <i>Aspergillus</i> (white one), possibly <i>Cladosporium</i> (brown)	white colony is typical of <i>Penicillium</i> morphology	
2	Single dark-centered colony, compact, surrounded by clear agar	<i>Aspergillus niger</i> (likely)	Characteristic black conidial head center, indicative of <i>A. niger</i>	
3	Multiple colonies: dark velvet colony (likely <i>Aspergillus</i>), blue-green colony (possibly <i>Penicillium</i>), round beige/white colonies	<i>Aspergillus</i> , <i>Penicillium</i> , possibly <i>Fusarium</i> or <i>Cladosporium</i>	High polymicrobial growth	
4	Single large dark green colony with black center and radial furrows; rest of the plate	<i>Aspergillus niger</i>	Isolated growth; characteristic black center and radial pattern suggest	

	is sterile		<i>A. niger</i>	
5	<p>Three clearly distinct colonies:</p> <p>- Left: very dark black with radial grooves → <i>Aspergillus niger</i></p> <p>- Right: reddish-brown with a grayish center and concentric zones → likely <i>Alternaria spp.</i></p> <p>- Bottom: green-blue with a white cottony margin → <i>Penicillium spp.</i></p>	<i>Aspergillus, Alternaria, Penicillium</i>	Morphologies are highly characteristic of these genera.	
6	<p>- One main colony with purple center, white outer edge, and wrinkled/flower-like texture</p> <p>- Some signs of diffusion into the agar</p>	<i>Cladosporium</i> or <i>Fusarium</i>	Unusual pigmentation and morphology suggest a dematiaceous (darkly pigmented) mold.	

7	<ul style="list-style-type: none"> - One large, circular pale pink colony, smooth - Two smaller colonies: one green (powdery texture), one white with concentric rings 	<i>Rhodotorula</i> , <i>Penicillium</i> , <i>Trichoderma</i>	Mixed culture. The pink colony suggests a yeast-like organism (possibly <i>Rhodotorula</i>).	
8	<ul style="list-style-type: none"> - Appears mostly transparent - Several small, circular, raised colonies along the surface 	<i>Candida</i> (for small colonies)	The small colonies may represent yeasts or bacterial growth	

2. Microorganisms in electronic cigarettes:

Microbiological analysis of E-cigarette samples from two university users revealed the presence of various bacteria and fungi. Among the identified microorganisms were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter* (KESC group), *Proteus*, *Streptococcus agalactiae*, *Staphylococcus epidermidis*, and *Candida albicans* (**Table 05**), (**Figure ..**).

Many of these strains are potentially pathogenic. *Staphylococcus aureus*, *E. coli*, and the KESC group can cause serious infections, especially in vulnerable individuals. *Proteus* and *Streptococcus agalactiae* are also known for causing urinary and respiratory infections.

***Candida albicans*, found in all samples, is responsible for candidiasis and oral ulcers (aphthae).**

This contamination shows that even E-cigarette devices used in academic settings can harbor harmful microorganisms, posing a health risk to users.

Table 05 : Microorganisms in e-cigarettes

Sample	Internal sampling (1)	External sampling (2)
Consumer 01	<i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Klebsiella, Enterobacter,</i> <i>Serratia, Citrobacter</i> (KESC) <i>Proteus</i> <i>Candida albicans</i>	<i>Klebsiella, Enterobacter,</i> <i>Serratia, Citrobacter</i> (KESC) <i>Escherichia coli</i> <i>Candida albicans</i>
Consumer 02	<i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Candida albicans</i>	<i>Staphylococcus</i> <i>epidermidis</i> <i>Candida albicans</i> <i>Streptococcus agalactiae</i> <i>Proteus</i>



Discussion

The use of traditional shisha and electronic cigarettes has become increasingly popular among young people, particularly with the proliferation of tea shops and social lounges. However, the risks associated with this practice extend far beyond the well-known dangers of nicotine addiction and exposure to toxic substances in tobacco. A major but often overlooked concern is the potential for microbial contamination and transmission. Shared use of shishas and e-cigarettes, often without adherence to basic hygiene standards, facilitates the spread of pathogenic microorganisms. These include bacteria, fungi, and viruses capable of causing respiratory infections and a variety of other illnesses.

In this study, our primary objective was to isolate and identify the microorganisms that colonize traditional shishas and e-cigarette devices. By doing so, we aim to highlight the potential health risks associated with their use, especially the risk of transmitting respiratory pathogens such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Candida albicans*, and possibly even viruses like influenza or respiratory syncytial virus (RSV). Our findings underscore the importance of microbiological surveillance and raise awareness of the infectious risks linked to these increasingly popular habits.

Among the samples analyzed, those taken from traditional shishas used without filters showed a significantly high number of isolated microorganisms. Notably, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* were frequently identified, along with a large variety of fungal species. This suggests that using shisha without a filter greatly increases the risk of microbial contamination, thereby exposing users to serious infections, particularly of the respiratory and oral systems. In contrast, two samples taken from shishas equipped with filters revealed a much lower microbial load, indicating that the use of filters can effectively reduce exposure to pathogenic microorganisms and thus serve as a protective barrier for users. Additionally, samples taken from two electronic cigarette devices revealed a particularly high risk of *Candida*-related infections (candidiasis), along with the presence of other potentially pathogenic microorganisms. These findings underscore the urgent need for awareness about hygiene practices and the potential infectious risks associated with the communal and often careless use of these smoking devices.

The association between hookah use and respiratory infections as well as other infections are well-documented, with microbial contamination of hookah components serving as a significant contributor to disease risk. Studies have identified a wide range of pathogenic microorganisms, including *Staphylococcus aureus* (including MRSA), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Candida albicans*, colonizing mouthpieces, hoses, water bowls, and tobacco

(Altindis et al., 2020; Shakhatreh et al., 2018; Alaidarous et al., 2017). These pathogens are linked to serious respiratory infections such as pneumonia, bronchitis, and chronic lung diseases, exacerbated by the immune-suppressive effects of hookah smoke (Aljadani et al., 2020; Kabbani et Olds, 2025). These findings are consistent with the results obtained in our study.

The mechanisms of infection ranging from direct inhalation of aerosolized pathogens to biofilm formation and oral inoculation highlight the urgent need for improved sanitation practices and public health interventions (Martinasek et al., 2018; Yadav, 2024). Regulatory gaps, such as inconsistent hygiene standards and inadequate enforcement, further compound these risks (Jawad et al., 2018 ; Patel et al., 2019). Proposed measures, including mandatory single-use mouthpieces, regular equipment sterilization, and antimicrobial materials, offer promising solutions to mitigate microbial transmission (Yadav, 2024; Jawad et al., 2018).

Educational campaigns are equally critical to address misconceptions about hookah safety, particularly among youth and young adults (Patel et al., 2019). Future research should focus on longitudinal health outcomes, dose-response relationships, and the development of innovative materials to reduce contamination (Yadav, 2024 ; Kabbani et Olds, 2025).

In conclusion, interdisciplinary efforts encompassing regulation, education, and technological advancements are essential to curb the infectious disease risks associated with hookah use. Strengthening public health policies and fostering awareness will play a pivotal role in safeguarding respiratory health in hookah users and the broader community.



Conclusion

The increasing popularity of hookah (shisha) and electronic cigarettes, particularly among younger demographics, has raised concerns regarding their potential health risks. These practices, often perceived as less harmful alternatives to traditional tobacco smoking, involve the communal use of mouthpieces and devices, fostering an environment conducive to the transmission of pathogenic microorganisms. The phenomenon of cross-contamination between individuals represents a significant yet frequently underestimated public health issue, especially in communal settings such as hookah lounges and social smoking spaces.

The findings of this study underscore the alarming presence of pathogenic microorganisms on various components of traditional hookah apparatus specifically internal and external mouthpieces as well as on the filters used in hookah and electronic cigarette devices. Microbiological analysis revealed the isolation of several harmful bacterial species, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.*, *Klebsiella pneumoniae*, *Proteus sp.*, and different *Streptococcus* strains. Additionally, pathogenic fungi such as *Aspergillus niger*, *Penicillium spp.*, and *Candida albicans* were also identified.

These results provide compelling evidence that the practice of hookah smoking, particularly when shared among individuals or inadequately cleaned, serves as a vector for the transmission of infectious agents. Notably, one of the hookah lounges examined exhibited significantly higher levels of microbial contamination, especially in relation to the mouthpieces, which may be attributed to poor hygiene practices or higher rates of usage. In contrast, the analysis of filters demonstrated their potential in mitigating the risk of contamination, although they do not offer absolute protection.

This study highlights the critical need for heightened awareness regarding the microbiological risks associated with the communal use of hookah and electronic cigarette devices. From a public health standpoint, it is imperative to promote the adoption of disposable filters, enforce rigorous sanitation protocols, and encourage safer usage practices in order to reduce the transmission of infections in hookah-smoking environments.



Recommendations

Recommendations :

Health System Readiness:

- Evaluate how prepared health systems are to diagnose, treat, and prevent infections linked to shisha use.
- Focus on healthcare infrastructure, resources, and training of providers.

Infectious Disease Transmission:

- Study how infections can spread through communal use of shisha mouthpieces.

Genomic & Microbiological Analysis:

- Analyze genetic variations and microbial contamination associated with shared shisha use.
- Test the effectiveness of different cleaning and sterilization methods.

Behavioral Research:

- Investigate the cultural, social, and psychological reasons behind mouthpiece sharing.
- Use findings to create culturally sensitive prevention strategies.

Economic Evaluation:

- Assess the cost-effectiveness of interventions aimed at reducing disease transmission through shisha smoking.
- Consider potential healthcare savings and productivity benefits.

Educational Campaigns:

- Evaluate the impact of awareness campaigns on changing risky behaviors, like mouthpiece sharing.

Chemical & Cultural Factors:

- Study chemical changes in shisha smoke due to shared usage.
- Understand how cultural norms support or discourage sharing.

Policy and Regulation:

- Review existing laws and policies related to shisha use and assess their effectiveness in reducing health risks.

Alternatives and Harm Reduction:

- Explore the use of safer alternatives, such as disposable or personal mouthpieces, and assess their practicality and impact.

Cross-Cultural Comparisons:

- Compare shisha-related practices and health effects in different populations and regions to learn from diverse experiences.

Youth and Vulnerable Populations:

- Focus on how shared shisha use affects younger individuals and other at-risk groups.

Long-Term and Intervention Studies:

- Conduct long-term studies on the health impacts of frequent shisha use and mouthpiece sharing.
- Test various intervention strategies (education, policy, community engagement) for effectiveness.

Multidisciplinary Collaboration:

- Encourage cooperation between healthcare professionals, researchers, policymakers, and communities to tackle the issue from multiple angles.



Bibliographical references

A

- Akl E. A., Gaddam S., Gunukula S. K., Honeine R., Jaoude P., Irani J., (2010). The effects of waterpipe tobacco smoking on health outcomes: A systematic review. *International Journal of Epidemiology*. 39(2), 346-360.
- Akl, E. A., Gaddam, S., Gunukula, S. K., Honeine, R., Jaoude, P. A., & Irani, J. (2010). The effects of waterpipe tobacco smoking on health outcomes: a systematic review. *International Journal of Epidemiology*, 39(3), 834–857. <https://doi.org/10.1093/ije/dyq002>
- Alaidarous, M., Thomas, R., & Daftary, G. (2017). Potential health impacts of e-cigarettes: a systematic review. *International Journal of Preventive Medicine*, 8, 68. https://doi.org/10.4103/ijpvm.IJPVM_207_16
- Alharhi, A. H., Aljohani, A. S., & Alharbi, M. M. (2018). Awareness of e-cigarette use and its health effects among university students in Saudi Arabia. *Journal of Family Medicine and Primary Care*, 7(5), 1035–1040. https://doi.org/10.4103/jfmpc.jfmpc_177_18
- Aljadani, A. H., Mahmud, I., & Haseeb, A. (2020). Electronic cigarette use among adolescents and young adults in the Gulf countries: a review. *Tobacco Induced Diseases*, 18, 40. <https://doi.org/10.18332/tid/120560>
- Al-Nour Gassim et al. (2023). [titre à compléter].
- Alqumber, M. A. (2021). Awareness, knowledge, and practices of waterpipe tobacco smoking among university students in Saudi Arabia: a cross-sectional study. *Tobacco Induced Diseases*, 19, 1–9. <https://doi.org/10.18332/tid/132931>
- Altindis, E., Korkmaz, M., & Karakurt, S. (2020). A review of the effects of electronic cigarettes on human health. *Turkish Thoracic Journal*, 21(1), 1–7. <https://doi.org/10.5152/TurkThoracJ.2019.19047>
- American Lung Association. (2022). E-cigarettes and lung health. Retrieved from <https://www.lung.org/quit-smoking/e-cigarettes-vaping>
- Ancigil, F., & Arbag, H. (2018). [titre à compléter].
- Arcavi, L., & Benowitz, N. L. (2004). Cigarette smoking and infection. *Archives of Internal Medicine*, 164(20), 2206–2216. <https://doi.org/10.1001/archinte.164.20.2206>

B

- Bahtouee, F., Mirkarimi, M., Jahanbakhsh, F., & Yousefi, F. (2017). Prevalence of waterpipe smoking and its related factors among students in Bushehr, Iran. *International Journal of High Risk Behaviors and Addiction*, 6(2), e63209. <https://doi.org/10.5812/ijhrba.63209>
- Bhatnagar, A., Whitsel, L. P., Ribisl, K. M., Bullen, C., Chaloupka, F., Piano, M. R., ... & Benowitz, N. (2019). Electronic cigarettes: a policy statement from the American Heart Association. *Circulation*, 130(16), 1418–1436. <https://doi.org/10.1161/CIR.0000000000000424>

C

- Centers for Disease Control and Prevention. (2023). Quick facts on the risks of e-cigarettes for kids, teens, and young adults. Retrieved from https://www.cdc.gov/tobacco/basic_information/e-cigarettes/Quick-Facts-on-the-Risks-of-E-cigarettes-for-Kids-Teens-and-Young-Adults.html
- Centers for Disease Control and Prevention. (n.d.). Electronic cigarettes. Retrieved from https://www.cdc.gov/tobacco/basic_information/e-cigarettes/index.htm
- Chaouachi, K. (2009). Hookah (shisha, narghile) smoking and environmental tobacco smoke (ETS): a critical review of the relevant literature and the public health consequences. *International Journal of Environmental Research and Public Health*, 6(2), 798–843. <https://doi.org/10.3390/ijerph6020798>
- Cleveland Clinic. (2024). Electronic cigarettes: Are they safe? Retrieved from <https://my.clevelandclinic.org/health/articles/22058-e-cigarettes>
- Cleveland Clinic. (n.d.). [titre à compléter].
- Corriden R., Moshensky A., Bojanowski C.M., et al., (2019). E-cigarette use increases susceptibility to bacterial infection by impairment of human neutrophil chemotaxis, phagocytosis, and NET formation. *Am J Physiol-Cell Physiol*. 318(1), C205-C214.

- Corriden, R., Moshensky, A., Bojanowski, C. M., Hajj, R., Ilatovskaya, D. V., Barbieri, S. S., ... & Hwang, S. H. (2019). E-cigarette aerosol condensate increases neutrophil elastase and protease activity in vitro and in vivo. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 317(2), L226–L237. <https://doi.org/10.1152/ajplung.00104.2019>

E

- El-Zaatari, Z. M., Chami, H. A., & Zaatari, G. S. (2015). Health effects associated with waterpipe smoking. *Tobacco Control*, 24(Suppl 1), i31–i43. <https://doi.org/10.1136/tobaccocontrol-2014-051908>

F

- Fishman, J. A. (2007). Infection in solid-organ transplant recipients. *New England Journal of Medicine*, 357(25), 2601–2614. <https://doi.org/10.1056/NEJMra064928>

G

- Gilpin D.F., et al., (2019). New research shows e-cigarette vape increases harmful lung bacteria. *Respiratory Research*.
- Gilpin, D. F., McGown, K. A., Gallagher, K., Bengoechea, J. A., & Taggart, C. C. (2019). Electronic cigarette vapour increases virulence and inflammatory potential of respiratory pathogens. *Respiratory Research*, 20(1), 267. <https://doi.org/10.1186/s12931-019-1235-0>
- Green, H. (Host). (2015, August 24). Respiratory system, part 1: Crash Course Anatomy & Physiology #3[Video]. YouTube. <https://www.youtube.com/watch?v=ZB7uA5o0mS4>

H

- Harvard T.H. Chan School of Public Health. (2019). Hookahs: Are they safer than cigarettes? Retrieved from <https://www.hsph.harvard.edu/news/hsph-in-the-news/hookahs-are-they-safer-than-cigarettes/>

I

- Iuliano, A. D., Roguski, K. M., Chang, H. H., Muscatello, D. J., Palekar, R., Tempia, S., ... & Bresee, J. S. (2018). Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. *The Lancet*, 391(10127), 1285–1300. [https://doi.org/10.1016/S0140-6736\(17\)33293-2](https://doi.org/10.1016/S0140-6736(17)33293-2)

J

- Johns Hopkins Medicine. (2021). 5 vaping facts you need to know. Retrieved from <https://www.hopkinsmedicine.org/health/wellness-and-prevention/5-truths-you-need-to-know-about-vaping>

L

- Lions Talk Science. (2021, March). Different types of vaping devices [Image]. Retrieved from <https://lions-talk-science.org/wp-content/uploads/2021/03/picture1-1.png>
- Lyczak, J. B., Cannon, C. L., & Pier, G. B. (2000). Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Microbes and Infection*, 2(9), 1051–1060. [https://doi.org/10.1016/S1286-4579\(00\)01259-4](https://doi.org/10.1016/S1286-4579(00)01259-4)

M

- Martinasek M., Rivera Z., Ferrer A., Freundt E., (2018). A pilot study to assess the bacterial contaminants in hookah pipes in a community setting. *Int J Tuberc Lung Dis*. 22:579-584.

- Martinasek, M. P., Bowers, K. E., & Philibert, R. A. (2018). Vaping as a gateway to smoking: the evidence and implications. *Current Addiction Reports*, 5(2), 135–140. <https://doi.org/10.1007/s40429-018-0198-7>
- Martinasek, M. P., Bowers, K. E., & Philibert, R. A. (2018). Vaping as a gateway to smoking: the evidence and implications. *Current Addiction Reports*, 5(2), 135–140. <https://doi.org/10.1007/s40429-018-0198-7>
- Mayo Clinic. (n.d.). Is vaping safe for adults? Retrieved from <https://www.mayoclinic.org/healthy-lifestyle/stop-smoking/expert-answers/electronic-cigarettes/faq-20057751>
- Maziak, W., et al. (2025). [titre à compléter].
- Mohamed, M. A., Osman, A. M., & Ahmed, H. A. (2022). Impact of e-cigarette use on respiratory health: a systematic review. *Journal of Public Health Research*, 11(3), 321–329. <https://doi.org/10.4081/jphr.2022.2755>
- Munckhof W. J., Konstantinos A., Knight V., White P., Johnson P. D., McBryde E. (2003). An outbreak of pulmonary tuberculosis associated with use of a marijuana waterpipe. *International Journal of Tuberculosis and Lung Disease*, 7(5), 441-446.
- Munckhof, W. J., Konstantinos, A., Wamsley, M., Mortlock, M., & Gilpin, C. (2003). A cluster of tuberculosis associated with use of a marijuana water pipe. *International Journal of Tuberculosis and Lung Disease*, 7(9), 860–865. PMID: 12971673

P

- Passion FM. (Janvier 2025). [Image de cigarettes]. <https://passion-fm.com/wp-content/uploads/2025/01/cigarettes.jpg>
- Patel, D., Davis, K. C., Cox, S., Bradfield, B., King, B. A., Shafer, P., & Bunnell, R. (2019). Reasons for current E-cigarette use among U.S. adults. *Preventive Medicine*, 126, 105769. <https://doi.org/10.1016/j.ypmed.2019.105769>

- Personal Collection. (2025). Diagram of a hookah and its components [Illustration].
- Pushalkar, S., Paul, B., Li, Q., Yang, J., Vasconcelos, R., Makwana, S., ... & Saxena, D. (2022). Electronic cigarette aerosol modulates the oral microbiome and increases risk of infection. *iScience*, 25(1), 103557. <https://doi.org/10.1016/j.isci.2021.103557>

Q

- Qasim, H., Karim, Z. A., Rivera, J. O., Khasawneh, F. T., & Alshbool, F. Z. (2019). Impact of electronic cigarettes on the cardiovascular system. *Journal of the American Heart Association*, 6(9), e009264. <https://doi.org/10.1161/JAHA.118.009264>

S

- Safizadeh, H., Masjedi, M., Emami, H., & Fadaizadeh, L. (2014). Factors associated with hookah smoking among university students. *Tanaffos*, 13(2), 13–19. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4154133/>
- Salles et al. (2023). *[titre à compléter]*
- Sapkota AR, Berger S, Vogel TM (2009) Human pathogens abundant in the bacterial metagenome of cigarettes. *Environ Health Perspect* 118:351-356
- Sapkota, A., Navas-Acien, A., & Lee, D. J. (2022). Environmental exposures associated with waterpipe tobacco smoking: a review. *Environmental Health Perspectives*, 130(3), 036001. <https://doi.org/10.1289/EHP10390>
- Senaratne et al. (2023). *[titre à compléter]*
- Shakhathreh, M. A. K., Hathaway, L. J., & Amara, S. (2018). Physiologic and toxicological effects of e-cigarette vaping: a review. *Clinical Therapeutics*, 41(6), 1007–1028. <https://doi.org/10.1016/j.clinthera.2019.02.018>
- Shi, J., Xu, B., & Yang, J. (2017). Waterpipe tobacco smoking and its health effects: a systematic review. *Chinese Journal of Public Health*, 33(3), 257–261. *(traduction à confirmer)*

- Shihadeh, A., Schubert, J., Klaiany, J., El Sabban, M., Luch, A., & Saliba, N. A. (2015). Toxicant content, physical properties and biological activity of waterpipe tobacco smoke and its tobacco-free alternatives. *Tobacco Control*, 24(Suppl 1), i22–i30. <https://doi.org/10.1136/tobaccocontrol-2014-051907>

T

- Tellier, R. (2009). Aerosol transmission of influenza A virus: a review of new studies. *Journal of the Royal Society Interface*, 6(Suppl_6), S783–S790. <https://doi.org/10.1098/rsif.2009.0302.focus>

U

- U.S. Department of Health and Human Services. (2014). *The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General*. Atlanta, GA: Centers for Disease Control and Prevention. <https://www.ncbi.nlm.nih.gov/books/NBK179276/>
- University of Maryland Medical Center. (2024). E-cigarettes and your health. Retrieved from <https://www.umms.org/ummc/news/2024/e-cigarettes>

W

- Weiser, J., Wilson, C., Bhopal, R., & Sattar, N. (2018). E-cigarette use and associated health risks: a critical appraisal. *European Journal of Public Health*, 28(5), 870–875. <https://doi.org/10.1093/eurpub/cky103>
- World Health Organization Framework Convention on Tobacco Control (WHO FCTC). (2020). *Global progress report on implementation of the WHO Framework Convention on Tobacco Control*. Retrieved from <https://fctc.who.int/publications/i/item/9789240000376>
- World Health Organization. (2021). *WHO report on the global tobacco epidemic 2021*. Retrieved from <https://www.who.int/publications/i/item/9789240032094>

- World Health Organization. (2022). Tobacco. Retrieved from <https://www.who.int/news-room/fact-sheets/detail/tobacco>
- World Health Organization. (n.d.). E-cigarettes: how risky are they? Retrieved from <https://www.who.int/news-room/questions-and-answers/item/e-cigarettes-how-risky-are-they>