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Isolation and Diagnosis or Identified Microorganisms for Hookah in Najaf Province

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Abstract

Young adults who use hookahs continue to pose a risk to public health. The harmful effects of hookah smoking, which include both acute and chronic health concerns, have been revealed by more and more study. Hookah clubs and lounges lack procedures for equipment sanitation because there is currently no regulation in place. To investigate any indications of bacterial contamination in hookah pipes as a result of noncompliance with sanitation rules. Additionally, when mouthpieces is shared, there is an increased chance of contracting various respiratory illnesses, such as those caused by viruses, oral bacteria, and fungi. infection such as *E. coli*, *Mycobacteria tuberculosis*, *Pseudo monas Sp.*, *Staphylococcus aurous*, *Salmonella Sp.*, and *klebsella Sp.*; *Aspergillus Aspergillus flavus*, *niger*. *Rhizopus Sp.*, *Candida Sp.*, and *Curvularia Sp.* Researchers found that the use of hookahs has become a public health concern due to secondhand smoke, the spread of infectious diseases like TB, herpes, and Aspergillus infection, as well as chronic lung diseases like pulmonary TB, lung abscess, bronchopneumonia, with residual lung cavity, asthma, and lung malignancy that have been documented. Bacterial and Fungal sample were collected at 15 hookah bars in Najaf Province, at each of the 15-hookah bare, Afruit –flavored hookah was ordered and sterile Specimen were collected for bacterial and Fungi examination from four predetermined parts on the hookah pipe. Specifically, the sampled areas included the inside of the fixed mouthpiece, personal from mouth swabbed, detachable hose near the water vase and the non-diSposable hose connector where the adetachable hose is attached. These sites were sampled with asterile cotton swab,which was then applied to the surface of sterile DifcoTM. The samples were transfered Microorganisms to laboratory of advanced mycology \faculty of science \Kufa University for diagnosis and study.

Keywords: Isolation, Diagnosis, Identified Microorganisms, Hookah, tobacco

Introduction

Young people are increasingly congregating in hookah bars to socialize, particularly college students from middle-class and upper-class backgrounds (Jehi *et al.*,2024). A hookah is a water pipe with several hoses attached, and many people congregate at a hookah bar to smoke flavored tobacco (Martinasek *et al.*,2011). Each

participant uses a single mouthpiece to breathe in smoke from the device (Daher *et al.*,2010). An attached water bowl that is partly full with water is part of the hookah, or water pipe (Armenteros., 2020). The smoker inhales through a mouthpiece on the other side of the hose that

is attached to the top of the water bowl (Mandal *et al.*, 2021).

Before being inhaled through the mouthpiece, the smoke travels through the water (Stadnytskyi *et al.*, 2021). The smoke is cooled as hookah bubbles pass through water at the pipe's base (Mandal *et al.*, 2021). A hookah smoker must inhale twice as deeply as a cigarette smoker due to this chilling process, which deepens the smoke's penetration into the lungs (van der and Garfield, 2021).

The aim of the study was achieved through several key objectives, including the isolation and identification of bacteria and fungi associated with hookah and water pipe use. Water pipe smoking is recognized as a significant public health hazard and a global concern, yet its specific impact on dental health remains poorly understood. The study examined changes in the oral microbial flora resulting from water pipe smoking. Additionally, it highlighted the need for further investigation into the potential link between hookah bars and the transmission of tuberculosis (TB).

Its concentrated harmful from thought the hose (Khartoum) which is placed in the mouth and withdraws smokes (Dar-Odeh and Abu-Hammad., 2021). which containing that more than(19) chemical material are known structures and these products thermal analyses are linked with nucleic acid and thus causing cancer and many genetic mutations (Aljadani *et al.*.,2020) . where smoke (100) % cigarette smoke because of smoke does not pass through the filters such as cigarettes, but body needs directly (Wei *et al.*., 2024) and carrying with the toxins different types and thus a high rates toxins in blood about (26)%. The hookah water with filing some of the toxins in existing toxins in smoke (Badran and Laher ., 2020) . But the nicotine does not filtering water .As well as to not changing hookah water makes it wash water and environment live suitable for many of bacteria , fungi and parasites (Armenteros .,2020) . Usually, shisha smoking draws attention to the negative consequences of inhaling smoke (Mahmood *et al.*.,2024) . For example, the Centers for Disease Control cautions that "the hookah's use of charcoal to heat tobacco raises the health risks by producing smoke that contains high levels of metals, carbon monoxide, and chemicals that cause cancer (Mandal *et al.*., 2021) .

The traditional kinde known in communities That consist of the from the tobacco leaves raw dry that are moisture with water before using there is no flavoure in this type (Raman Manoharlal.,2023). Second type of hookah named the wordin flavoure sweet which derivative word of hone in Arabic and the kind flavoure and sweets manufactured this character with this kind of fermentation tobacco with pins and fruit Spirit (Lawrence ., 2019) .Saves the product moisture so that the time of used .and use the vegetables in the all kinds to boiling or burning the tobacco products that are placed in top of the hookah and lead to increased healthy risks (Braunstein ., 2022) . Although the negative health effects of smoking cigarettes are widely known, nothing is known about how smoking a waterpipe affects one's health (Arshad *et al.*, 2019). Prior research has demonstrated that the toxicant profile of smoke from a waterpipe is comparable to that of cigarette smoke, albeit with varying levels (Kaplan *et al.*, 2019). For instance, a single waterpipe session produces around five times as much tar as a single cigarette (Bhatnagar *et al.*, 2019). Similarly, smoking a waterpipe exposes you to at least many times as much carbon monoxide as smoking cigarettes (Rezk *et al.*., 2019) . Additionally, compared to cigarette smoke, waterpipe smoke contains many times more polycyclic aromatic hydrocarbons (Masjedi *et al.*., 2023) . Furthermore, smoking a waterpipe causes a significantly increased amount of smoke exposure, more tobacco use per smoking session, and longer smoke inhalation times (Qasim *et al.*., 2019) . Lastly, since tobacco in waterpipes is typically combined with tastes, sugar, and glycerol and burned by charcoal, it is anticipated that smoking a waterpipe will have a noticeable impact on the oral microbial flora (Shakhatreh *et al.*., 2018) .

Numerous hookah bars have different levels of bacterial contamination, which is indicative of a lack of industry standards for cleaning these equipment (Martinasek *et al.*., 2018) . Hookah pipe bacterial infection could be a gateway for the spread of infectious diseases (Qasim *et al.*., 2019) . Our findings support continued ongoing surveillance to look for possible human infections (Martinasek *et al.*., 2018) . According to research on the impact of smoking on oral health, smoking is linked to periodontal disease, oral infections, oral cancer, and disruption of normal flora and taste (Shakhatreh *et al.*., 2018) . Additionally, smokers were substantially more

likely than non-smokers to spread a number of oral infections, including *Tannerella*, *Porphyromonas gingivalis*, *Prevotella nigrescens*, and *Prevotella intermedia* (Enchiparambil ., 2022) . There aren't many research that look at how smoking a waterpipe affects dental health. According to a recent study, smoking a waterpipe is strongly linked to periodontal disease (Grillo *et al* ., 2022) . Waterpipe smoking has also been demonstrated to dramatically increase lower lip squamous cell carcinoma, keratoacanthoma, and potentially malignant oral mucosal ulcers (Szyfter *et al* ., 2019) .

Fungi are thought to be human disease agents, and deaths brought on by fungal diseases are frequently disregarded (Rodrigues and Nosanchuk ., 2021) .

Usually linked to underlying illnesses and medical treatments, opportunistic fungal infections can result in host immunodepression and anatomical barrier disruption (Brown *et al* ., 2024) Socioeconomic status, geographic location, cultural practices, and the proportion of people with risk factors for fungal infections all affect the occurrence of fungal infections (Jenks *et al* ., 2023) .Most healthy people's host epithelium surfaces are home to some fungus, but in immunocompromised people, they can cause potentially fatal systemic infections (Pathakumari *et al* ., 2020) .

Materials and methods.

The study was conducted using the flowing instruments and equipment listed in Table (1)

Table (1): Equipments and instruments used in this study

Equipment	Company/Origin
Autoclave	Sano Clave / Japan
Compound light microscope	Olympus / Japan
Digital camera	Sony / Japan
Distillator	Gel / Germany
Incubater	Sanyo/ Germany
Laminar flow cabinet	Lab TECH / Germany
Para films roll	Pechiney/ USA
Refrigerator	Concord / Lebanon
Balance that is sensitive	Sartorius / Germany
Slides as well as cover slides	BBL / USA

Table (2): Culture media for isolating bacteria and fungi.

Culture media	Manufacturing	Origin
(XLD)Xylose-Lysine medium	Oxoid	Paris ,France
Blood agar base	Oxoid	Paris ,France
Brain heart infusion broth	Oxoid	UK
Mac Conkey agar	Oxoid	Paris ,France
Muller-Hinton agar	Oxoid	UK
Nutrient agar	Oxoid	Paris ,France

Nutrient broth	Oxoid	Paris ,France
Salmonella Shigella agar	Oxoid	UK
Tryptic Soy broth	Himedia	India
Mannitol agar	Himedia	India
Congo red agar	Oxoid	UK
Patato dextrose agar	Himedia	India
Sabouraud's Dextrose	Himedia	Ndia

Samples characterization

The collected agar plates were inoculated and number is 15 specimens for 24-48hr .The plates were then observed for bacterial growth ,The individual colonies were selected and streaked for isolation on differertion media and which were then further characterized to determine cellular morphology ,gram reaction, growth on selective and differential ,catalase, coagulase reaction. The identity of select Gram-negative, oxidase-negative ,isolates was investigated using the Enteropluri test (BD,Franklin Lakes,NJ,USA).

Identification of bacteria

The following microscopic features were used to diagnose the bacteria isolates based on their culture.

Cultural and morphological traits

The isolates of bacteria were examined for their size, shape, color, edge, and appearance on N.A for 24 hr in 37°C and fungal growth on SDA media after 25±2 hr. of incubation for yeast isolates and 5-7 days for molds isolates A single cell was taken from the yeast growth on SDA and cultured using the loop method, which was incubated for 24 to 48 hours at 37°C. The chromagar test was used to assist diagnose the Candida species based on color (Bayona *et al.*, 2020).

Diagnosis and Isolation of Fungi

The diagnosis of isolated fungi was carried out as follow:

A. Growth colony morphological characteristics, such as color, texture, edge, colony reversal, and pigments produced (Pyrry *et al.*, 2021).

B. Using a light microscope to examine the morphologies of fungus, as well as their conidia and mycelium (Senanayake *et al.*, 2020).

Microscopic examination

To be purified and identified, the fungal isolates were moved to sterile plates. The cultivated fungi were put on a slide, covered with a cover slip, stained with lactophenol cotton blue to find fungal structures and gram stain to identify yeast, and then identified under a microscope based on their colony morphology and spore characteristics (Byrne and Rankin ., 2021).

Gram staining

After making streaks on alcohol-cleaning slides, the slides were heat-fixed by floating them in crystal violet solution for a minute and another minute in gram's iodine. After washing, 95% ethanol was used to decolorize the smears, and aqueous basic fuchsin was used to counter-stain them. The slide was next inspected with a bright field microscope's oil immersion objective lens (100 x) to check for the presence of bacteria and Candida morphology (Kulkarni *et al.*, 2021).

Results and Discussion

The smoker's mouth and mouthpiece showed the highest bacterial diversity and prevalence among the 15 hookahs analyzed, including Salmonella Sp., Pseudomonas, E. Coli, Staphylococcus aureus Sp., Klebsella Sp., and Mycobacteria Sp. It was discovered that a few of the bacterial isolates were resistant to antibiotics. Two of the recovered bacteria were found to be involved in hookah surveillance to keep an eye out for any human diseases, while 10 of the bacteria were Gram-

positive. When the yeast (such as *Candida Sp.*) is visible. In the same time we are appearance in second degree of waterbowl and Hose connector such as *E.coli*, *Pseudomonas Staphylococcus aureus Sp.*,The contaminated Microorganisms in several part of waterpipe was resulted The danger of contracting colds, fungus, oral bacterial infections, and other communicable diseases including tuberculosis increases when mouthpieces from hookah pipes are shared.

Additionally, because hookah waterbowls aren't replaced for a long period Numerous investigations into how smoking affects oral microbiota have revealed notable variations in the subgingival bacteria between smokers and non-smokers. For instance, according to Zambon, smokers have noticeably greater concentrations of the bactericides forsythias subgingivally (Shakhatreh *et al.*, 2018).

Table (3) Appearance bacteria isolation from personal smokers' mouth and hookah sample.

Samples	Bacteria isolation					
	<i>E. coli</i>	<i>Pseudomonas Sp</i>	<i>Staph .aureus</i>	<i>klebseilla Sp.</i>	<i>M. tuberculosis</i>	<i>Salmonella Sp.</i>
Smokers Mouth	<i>E. coli</i>	<i>Pseudomonas Sp</i>	<i>Staph .aureus</i>	<i>klebseilla Sp.</i>	<i>M. tuberculosis</i>	<i>Salmonella Sp.</i>
Mouthpiece	<i>E. coli</i>	<i>Pseudomonas Sp</i>	<i>Staph .aureus</i>	<i>klebseilla Sp.</i>	<i>M. tuberclosis</i>	<i>Salmonella Sp.</i>
Water Bowl	<i>E. coli</i>	<i>Pseudomonas Sp</i>	<i>Staph .aureus</i>	-	-	-
Hose Connector	<i>E. coli</i>	<i>Pseudomonas Sp</i>	<i>Staph .aureus</i>		-	-

As noted previously in specimen from more bar or coffe shops were contaminated with fungus that presence of *Asp . niger*, *Asp . flavus*, *Curvularia Sp.*, *Candidia Sp.* and *Rhizopus Sp.in flavoure sweet (flavoure tobacco)*.While we find *candidia Sp.* in mouth hookah smokers.On the other hand exist of the yeast and *Rhizopus Sp.in waterfowl* Table(4-1) *.E.coli*, *Mycobacteria tuberculosis*, *Pseudomonas Sp.*, *Staphylococcus aureus*, *Salmonella Sp.*,and; *Aspergillus*

niger, *Aspergillus flavus*, *Curvularia Sp.*, and *Candidia Sp.* Numerous investigations into how smoking affects oral microbiota have revealed notable variations in the subgingival bacteria between smokers and non-smokers. For instance, Zamboni found that smokers had noticeably greater subgingival concentrations of the bactericide Bactericides forsythias (Shakhatreh *et al.*, 2018).

Table (4) Funi isolation from personal smokers' mouth and waterpipe sample.

Samples	Fungi isolation				
	<i>Candidia Sp</i>	Yeast	-	-	-
Smokers Mouth	<i>Candidia Sp</i>	Yeast	-	-	-
Flavoure Sweet	<i>Asp. Niger</i>	<i>Asp. Flavus</i>	<i>Curvularia Sp.</i>	<i>Candidia Sp</i>	<i>Rhizopus Sp.</i>
Water Bowl	<i>Rhizopus Sp.</i>	Yeast	-	-	-

Additionally, it has been demonstrated that waterpipe smoking damages content cells' DNA (Alsaad *et al.*, 2019). Numerous anaerobic periodontal pathogens, including *P. gingivalis*, *Tannerella forsythia*, *P. intermedia*, *Eikenella corrodens*, *Campylobacter rectus*, *Aggregatibacter actinomycete mcomitans*, *Treponema denticola*, and *Fusobacterium nucleatum*, have been found to be present in the human oral cavity (Colombo and Tanner 2019). It has been demonstrated that smoking cigarettes changes the frequency of these bacteria in the oral cavity (Shakhatreh *et al.*, 2018). In this study, we examined the impact of waterpipe smoking on the profile of normal flora in the oral cavity and subgingiva, which is still unknown and unclear, because the toxicant profile and smoking behavior differ between waterpipe and cigarette smoking. Policies and treatments that target waterpipe smokers may be developed using the study's findings. Numerous investigations into how smoking affects oral microbiota have revealed notable variations in the subgingival bacteria between smokers and non-smokers. For instance, according to Zambon, smokers had noticeably greater subgingival *Bacteroides forsythus* levels (Shakhatreh *et al* 2018).

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