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ORIGINAL ARTICLE

Microbial contamination in the communal-use Lao tobacco waterpipe

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Background: The use of the Asian tobacco waterpipe (TWP) in the Lao People's Democratic Republic represents a potential communal source of infectious disease. This practice of smoking can lead to weakened defences of a smoker's respiratory epithelium, making the smoker vulnerable to respiratory diseases such as coronavirus disease 2019, tuberculosis and others.

Methods: This study evaluated the water quality and hygiene factors among 43 smokers of five villages in rural Luang Namtha Province. Water samples were collected from participant's TWPs and assessed for the presence of *Escherichia coli*, coliforms and aerobic plate count (APC) bacteria using the 3M Petrifilm.

Results: The microbial indicator testing results were 95% positive for the APC, 38% positive for coliforms and 17% positive for the *E. coli* indicator. The concentrations were highest for the APC, with an average of 10^6 colony forming units (cfu)/ml, followed by coliforms with <100 cfu/ml and lowest for *E. coli* with <10 cfu/ml. Most TWPs were infrequently cleaned, heavily used and contained a warm, brown-coloured water.

Conclusions: The warm, dark and moist internal water container may facilitate microbial survival and growth. The use of a TWP adds several unstudied modes of transmission to a complex and common biobehavioural and environmental pathogen exposure. Future TWP cessation activities should be tailored to consider risks of infectious disease transmission.

Keywords: infectious disease, tobacco waterpipe, tuberculosis, water quality.

Introduction

Tobacco users in Asia are 4.5 times more likely to die from tuberculosis (TB) than non-smokers due to the weakened defences of a smoker's respiratory epithelium.[1] In addition to TB, tobacco use can also substantially increase the chance of adverse health outcomes due to coronavirus disease 2019 (COVID-19) infection[2] as well other infectious disease exposures[3,4] and other respiratory viruses.[5]

The use of a tobacco waterpipe (TWP) represents a communal source of infectious disease that is common in many Southeast Asian and Middle Eastern countries. The Asian TWP is an alternative method to smoke tobacco[6] in the Lao People's Democratic Republic (Lao PDR or Laos). Several ethnic minority groups and rural communities in Southeast Asia use simple homemade bamboo TWPs that allow a smoker to inhale combustible tobacco products through a reservoir of water (Figure 1). It is growing in popularity and there are now an estimated 4.1 million TWP smokers in Vietnam.[7] A national survey among ethnic minorities living on the Laos–Cambodia border showed 30.1% of men and 48.6% of women are TWP smokers.[8] In rural northern Laos, our team found that 97% of TWP smokers actively share their TWPs and rarely clean them.[6] This presents an opportunity for disease transmission through the communal use of TWPs.

More than 97% of TWP smokers in rural northern Laos report sharing their homemade bamboo TWPs with other smokers. The social use of the TWP[9] in Laos is similar to the estimated 90% of TWP smokers who share their 'hookah' pipes in the Eastern Mediterranean region and many western areas[10] (Figure 1). The communal use behaviours associated with the Asian TWP are a public health concern that represent transmission of infectious pathogens including herpes simplex virus, Epstein–Barr,[11] *Helicobacter pylori*,[3] hepatitis B, various respiratory viruses, bacterial meningitis,[12] periodontal conditions such as oral candida[13,14] and TB.[13–15] Most Asian TWP smokers do not routinely clean their TWPs;[6] the dark, warm, humid and nutrient-rich internal

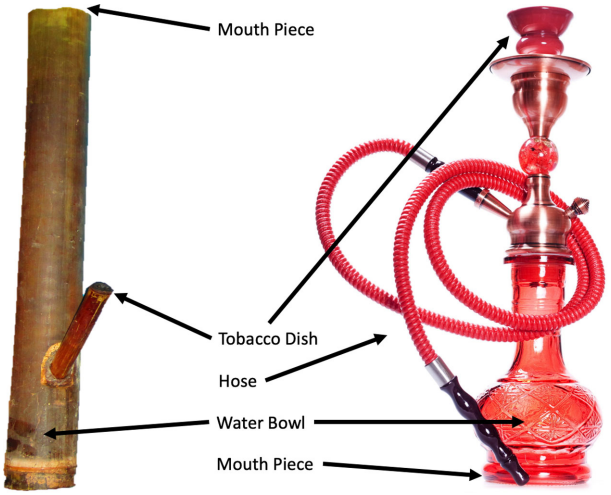

Figure 1. Characteristics of the Asian (left) and Eastern Mediterranean (right) waterpipe.

components can promote survival of pathogens outside of the host. There are also several culture-independent 16S ribosomal RNA gene sequence analysis studies on tobacco and many classes of microorganisms have been identified in cigarettes, including potentially pathogenic microbes within the genera *Actinetobacter*, *Bacillus*, *Clostridium*, *Enterococcus*, *Klebsiella*, *Pseudomonas* and *Staphylococcus*,[16–19] with a recent study investigating the microbes present in Eastern Mediterranean waterpipes[19] that identified common environmental species that could cause human disease in rare cases.

Although many of the above case reports and product contamination studies show the potential for contamination, no project has yet to document cultivatable bacteria in the water of Lao TWPs in a resource-poor setting. Overall, the majority of published health concerns from Asian TWPs are typically regarding non-infectious disease; those are discussed in the mixed methods report from this group's Laos study (Table 1) and previous reports from this group's research in the Western Pacific region.[6,8,20] This study investigates the occurrence of bacteria in TWPs and the potential for disease transmission. The field study performed a microbial water quality assessment from actual TWPs recently smoked in Luang Namtha Province of rural northern Laos.

Materials and methods

Samples of water from the TWPs were assessed for *Escherichia coli*, aerobic plate count (APC) bacteria and coliform indicators in TWPs from four rural Lao communities in rural Luang Namtha Province. The four communities were selected through consultation with the Lao Ministry of Health, who were already working with the research team on the Lao national tobacco survey.[21] The study site of Luang Namtha was recommended because there is a high prevalence of TWP smokers and a supportive community organization, the Adventist Development Relief Agency (ADRA). The ADRA provided access to their network of community leaders and local project partners. The study was conducted during July 2011 in four villages of Luang Namtha Province: Luang Namtha (N20.967554, E101.404850), Oudong Sim (N21.005854, E101.408961), Thong Om (N20.9837322, E101.406237) and Muang Sim (N21.189988, E101.151326).

The study team worked with multilingual interviewers and the local village chiefs to complete a convenience sampling of TWP smokers. The selection criteria included age ≥18 y who allowed investigators to examine the TWP that they last smoked. Oral informed consent was obtained from the participants that the water in their TWPs would be sampled for bacteria. Ethics approval was obtained from the National Ethics Committee for Health Research located in Vientiane and the Institutional Review Board of Loma Linda University.

The study participants were recruited by their village chiefs and representatives of the ADRA. The volunteer participants gathered in village schools with their TWPs.

Participants were asked to provide their TWPs so members of our team could obtain a 200-ml water sample. The TWP was lightly swirled for 10 sec and then the water was poured through

Table 1. Results from a previous article[6] detailing the demographic and behavioural variables from the study population

Variables	Values		
Gender, n (%)	Male, 39 (90.7)	Female, 4 (9.3)	
Read/write, n (%)	Yes, 12 (29.3)	No, 29 (70.7)	
Career, n (%)	Government, 1 (2.4)	Farmer, 33 (78.6)	Homemaker, 7 (16.7)
Type of TWP, n (%)	Bamboo, 39 (92.9)	Metal, 2 (4.8)	PVC, 1 (2.4)
Own TWP, n (%)	Own and will share, 41 (97.6)	Own and will not share, 1 (2.4)	
Age (years), mean ± SD	49 ± 13.8		
Number of persons who shared the TWP during the last smoking session, mean ± SD	1.21 ± 0.54		
Number of persons who shared the TWP during the last week, mean ± SD	5.24 ± 3.9		

SD: standard deviation.

Figure 2. A field laboratory technician pouring water from the TWP into a sample collection bottle.

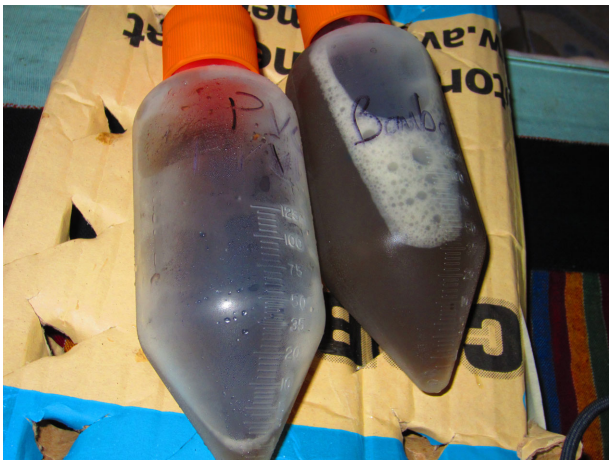

Figure 3. The appearance of water in most of the TWP water samples.

the stem (bowl) of the TWP and into a sterile 250-ml sample container. In one village, the TWP smokers preferred pouring water through the stem of the device rather than the mouthpiece. In all other villages the water was poured through the mouthpiece, as shown in Figure 2. The TWP samples were labelled with the date, village number, TWP type and gender of the smoker. The samples were stored on ice and processed within 6 h of collection.

The samples were taken to a central location and processed using the 3M Petrifilm *E. coli*/coliform count plate and the 3M Petrifilm APC plates (3M, St. Paul, MN, USA) in a dual-chamber incubator (Millipore, Billerica, MA, USA). The APC is the 3M equivalent measurement of the heterotrophic plate count bacteria test that is a common water quality test used by the World Health Organization.[22] The 200-ml samples were split into duplicates, diluted using 10-fold dilutions and processed with the two types of Petrifilm in duplicate for each dilution. A dehydrated phosphate buffer solution (Fisher Scientific, Carlsbad, CA, USA) was combined with sterile water at room temperature and the pH was confirmed with Fisherbrand pH strips to be within a pH of 7–8 (Fisher Scientific). The dilutions were made in the phosphate buffer water using 10-ml sterile red-top glass vacutainers (with no additives) available from a pharmacy in Vientiane. The *E. coli* and coliform were incubated at 37°C for 24 h while the APC Petrifilm was incubated at 30°C for 72 h. A positive control, a trip blank and a laboratory-negative control were run for each day of sampling on the *E. coli*-specific Petrifilm using a lab strain of *E. coli* (ATCC 15597). The Petrifilm was counted and recorded in a project notebook with a maximum count of 150 colonies for the *E. coli* plates and 300 for the APC plates, while values above that were labelled by the team as 'too many to count' (TMTC). The ambient temperature and the TWP water temperature were collected before sampling with a glass thermometer. The pH of the TWP water was taken during sampling using Fisherbrand plastic pH strips (Fisher Scientific). Results of the cultivatable assays were entered into an Excel 2011 for Mac spreadsheet (Microsoft, Redmond, WA, USA) to organize data and to calculate means of replicates, geometric means, arithmetic averages, standard deviations and percent positives.

Table 2. Results of field water quality assessment from TWPs in rural Luang Namtha

Assessment	APC	Coliform	*E. coli*
Total samples, n	20	42	42
Positive, n	19	16	7
Positive, %	95	38	17
Non-detects, n	1	25	35

Results

The microbial indicator testing results were 95% positive for the 3M Petrifilm APC, 38% positive for coliforms and 17% positive for the *E. coli* indicator. The concentrations were highest for the APC, with a geometric mean of 10^4/ml and an average of 10^6/ml (Figure 4). The maximum coliform counts were <100/ml and the maximum *E. coli* counts were all <10/ml. The Lao drinking water recommendations state that *E. coli* and coliform counts should not be >0, meaning that none of this bacteria should be detected in drinking water.[23]

The appearance of the TWPs and the water inside was not clean. All TWPs except for three newly constructed TWPs had accumulated a black soot-like material on the inside of the TWPs. The TWPs' water appeared uniformly brownish tan in colour and had an odour of tobacco (Figure 3). The TWPs' internal water temperature was in the range of 27–30°C throughout the assessment. Some water samples that had no detectable APCs still had a brownish tan colour.

Overall, most of the water samples were contaminated with APC bacteria. These APCs, and the similar heterotrophic plate counts (HPCs), represent a large group of microbes that have no direct relationship with a health risk but are considered to be indicators of overall bacterial contamination.[22] The APC group represents bacterial contamination and includes many classes of bacteria, some of which may be opportunistic microbes that could infect the immunocompromised. A total of 17% of the TWPs were positive for *E. coli*, indicating faecal bacteria (Table 2). There was no source of treated drinking water in the villages, so the bacteria

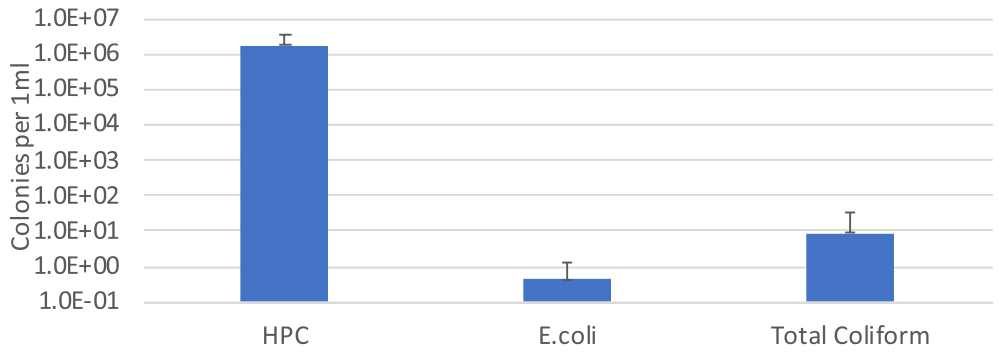

Figure 4. Average concentrations of indicator bacteria in TWPs in rural Luang Namtha, with an upper standard deviation line.

Figure 5. Some of the TWPs collected in one village during the study including bamboo, PVC and aluminium TWPs.

could have originated from the source water or from the constant exchanges on the mouthpieces of the TWPs.

Discussion

These results show that TWP smokers in Laos are exposed to an additional amount of environmental bacteria that has the potential to contribute to their infectious disease burden. The relationship of smoking with TB is now established[1] in Southeast Asia and the environment in these TWPs may allow further microbial survival of *Mycobacterium* or similar pathogens. Although there are limited infectious disease data available, the Lao government recognizes that TB is a major concern in rural and remote areas of Laos because of the population's limited access to TB services.[9] Infectious disease exposure should be considered in concert with the overall harm that the TWP smoker experiences[20] from non-microbial mechanisms. The recent COVID-19 pandemic presents a new challenge beyond TB, as the virus can also survive in dark, humid environments and is likely spread through communal use during the smoking process. In Laos, TWPs are occasionally made of materials other than bamboo. Figure 5 shows an aluminium TWP and one polyvinyl chloride TWP that were photographed at one of the three villages we visited. The varying surface types has direct implications in the survival of viruses, as new data show that COVID-19 may survive longer on plastics and metals than other materials such as cardboard.[24]

The team expected to find higher concentrations of *E. coli* and coliforms in the Lao TWPs because of the high reported amounts of contaminated water and the physical appearances of the TWPs. A 2017 national United Nations Children's Fund survey on health and environment[23] stated that 81% of all drinking water samples and 93% of all source waters in Luang Namtha Province of Laos were contaminated with *E. coli*. That survey reports a higher percentage of *E. coli* in drinking water sources than what this study found in the highly turbid TWP water, suggesting that later studies could use a different water quality indicator, as there are potentially several types of bacteria growing in the TWP water, as evidenced by the high APC concentrations.

Another reason why the team expected higher *E. coli* amounts is because the TWPs appear to be heavily used and soiled and contained warm water sourced from a river or open surface water. The TWPs were reported to be infrequently cleaned, with high moisture levels, a rough internal bamboo surface, a dark

environment for microbial survival and a potential supply of many sources of organic nutrients, as the user places his/her entire mouth on the TWP. These are all factors potentially contributing to the extended survival of Gram-negative bacteria. All of these hygiene and sanitation variables suggest that *E. coli* and coliform bacteria levels could be much higher. One potential consideration is that some tobacco has a minimal antimicrobial effect on some Gram-negative organisms.[25] More work is needed to assess the transport and survival of different classes of microbes in bamboo containers such as the TWPs smoked in Laos.

The demographic results of this study were reported earlier:[6] 90% of the participants were men, 69% had no formal education, 95% were married, 78% were farmers and 70% self-reported that they cannot read or write. A farmer's specific practices in rural Luang Namtha Province could put him at risk for additional respiratory health issues, as farmers work closely with domestic animals, conduct outdoor crop burning, actively burn manure indoors for a fuel source and use it as fertilizer.[26] The province of Luang Namtha was shown to have the highest reported use of pesticides across all of Laos in a United Nations Food and Agriculture Organization 2010 report.[27] Infectious disease vulnerability from TWP exposure could be further exacerbated by additional common practices such as indoor air pollution from using manure as a fuel and exposure to outdoor air pollution from crop burning practices.

The 2015 National Adult Tobacco Survey (NATS) in Laos found that most smokers had never received recommendations to quit smoking from a healthcare provider.[28] The potential for infectious diseases from smoking a TWP adds to the variety of potential tobacco-related illnesses that could initiate a tobacco cessation discussion by a healthcare provider. The 2015 NATS found that national smoking cessation activities were somewhat successful, but there was a need to tailor evidence-based cessation interventions to different subgroups throughout the country who are exposed to different types of tobacco use, such as the TWP in rural regions.

Conclusions

These pilot study findings indicate the possibility that TWP use adds several unstudied modes of transmission to a complex and common biobehavioural and environmental framework of pathogen exposure in rural Southeast Asia. This field assessment of water quality indicators demonstrates that the water inside Lao TWPs has the potential to be contaminated by several classes of microorganisms, but had a lower rate of *E. coli* contamination than province-wide water sources. The infectious disease burden of TWP use in this environment needs investigation in larger samples across larger panels of environmental pathogens. This will have implications for tobacco control, control of infectious diseases and community development.

Author's contributions: RS and PS contributed to the study design. RS, KS, RE and DS implemented the study. RS analysed the data, interpreted the results and wrote the manuscript. PS, KS, RE and DS assisted in formulating the manuscript to the final product. KS assisted in gaining approval from local and national government authorities. All authors read and approved the final manuscript.

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Competing interests: None declared.

Ethical approval: Approval of this study protocol was obtained from the Ethics Committee of the Ministry of Health in Vientiane and the Institutional Review Board of Loma Linda University.

Data availability: The data underlying this article will be shared on reasonable request to the corresponding author.

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