Bacteriological Comparison of Oral Swab Samples Collected from Conventional Cigarette Smokers and e-Cigarette Smokers

Submitted by

Indrajit Das
19326004

Istiak Sarwar
19326014

Plabon Ahmed
19326023

A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree with honors of
Bachelor of Microbiology

Mathematics and Natural Sciences Department
BRAC University
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Declaration

It is hereby declared that

1. The thesis submitted is my/our original work while completing the degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

Student's Full Name & Signature:

Indrajit Das 19326004	**Istiak Sarwar** 19326014

Plabon Ahmed
19326023

Approval

The thesis titled "[Bacteriological Comparison of Oral Swab Samples Collected from Conventional Cigarette Smokers and e-Cigarette Smokers]" submitted by

1. Indrajit Das (19326004)
2. Istiak Sarwar (19326014)
3. Plabon Ahmed (19326023)

of Fall, 2025 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of undergraduate on [16 November 2025].

Examining Committee:

Supervisor:
(Member)

_____

Nadia Sultana Deen

Associate professor, Mathematics and Natural Sciences Department

BRAC University

Program Coordinator:
(Member)

_____

Nadia Sultana Deen

Associate professor, Mathematics and Natural Sciences Department

BRAC University

Departmental Head:
(Chair)

_____

Md. Firoze H. Haque, PhD

Chairperson, Mathematics and Natural Sciences Department

BRAC University

Ethics Statement

Consent was taken from each individual (n=40) before taking an oral swab from them. Every single person had agreed to follow the criteria and contribute by providing their consent on the form given to them on the day of sample collection. They volunteered to give samples after filling out the consent form.

In this research, 8% similarities were found and 0% AI was found.

Abstract

This study explored the impact of Conventional Cigarette Smoker (CCS), Electronic Cigarette Smoker (ECS), and Conventional Cigarette Smoker+Electronic Cigarette Smoker (CCS+ECS) use on the oral microbiome in a population from Bangladesh, where clinical research on e-cigarette users remains scarce. Oral swab samples were collected from a total of 40 participants, including 10 e-cigarette users, 10 conventional cigarette smokers, 10 dual (E-Cigarette and Conventional Cigarette) users, and 10 non-smokers. Samples were cultured and Gram stained to identify the presence of Gram-positive and Gram-negative bacteria. In terms of culture-based study, Nutrient Agar (NA) medium and Blood Agar Plates (BAP) being used. The average total aerobic count on NA medium showed difference among the groups. The highest CFU/mL count were observed on CCS group (4.8×10^9 CFU/ml), followed by NS group (4.6×10^9 CFU/ml), CCS + ECS group (3.9×10^9 CFU/ml) and ECS (2.8×10^9 CFU/ml). In contrast, Blood Agar Plates (BAP), being enriched and capable of revealing hemolytic activity, provided an insight into bacteria that may be opportunistic or pathogenic whereas ECS showed the highest overall bacterial load, with BAP counts reaching 1.5×10^7 CFU/mL. CCS and CCS+ECS had comparable levels, ranging from 6.0 to 6.5×10^6 CFU/mL, while NS showed 7.7×10^6 CFU/mL. A significant difference between the ECS group, CCS group ($p = 0.02$) and CCS + ECS group ($p = 0.02$), indicating that e-cigarette users may harbor a greater abundance of fastidious bacterial species compared to other groups. It was hypothesized that smoking and vaping would disturb the natural oral microbial balance, increasing suspected harmful bacteria. In this case, we did the Gram-staining method and the findings revealed that CCS and ECS exhibited a higher presence of Gram-negative bacteria compared to other groups. CCS displayed a dominance of Gram-positive bacteria, suggesting a unique microbial response to tobacco exposure. ECS showed a slightly decreased Gram-negative count compared to NS, although the NS and CCS+ECS shared similar microbial profiles overall. Despite noticeable changes in bacterial composition, overall microbial diversity remained relatively stable, implying that host-related factors like genetics and hygiene may also have played a role. These results highlighted the compounded risks of e-cigarette and conventional cigarette use, questioning the idea of vaping as a more secure substitute to smoking. The pressing need for preventive health interventions and continued investigation of the long-term oral health outcomes of smoking and vaping was highlighted by the research, particularly in emerging markets such as Bangladesh.

Keywords: oral microbiome; e-cigarettes; conventional smoking; dual users; Gram staining; Bangladesh

Dedication

Dedicated to the Almighty and our Parents.

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List of Acronyms

BAP	Blood Agar Plates
NA	Nutrient Agar
CFU	Colony Forming Unit
NS	Non-Smokers
CCS	Conventional Cigarette Smokers
ECS	Electronic Cigarette Smokers
PEG	Polyethylene glycol

Chapter 1

1. Introduction

1.1 Background of the study

In recent years, the use of electronic cigarettes (e-cigarettes) has grown exponentially, particularly among young adults and former smokers who had sought substitutes for conventional cigarettes. Recently, the emergence and popularity of e-cigarettes and vapes have prompted worries about their potential impact on oral health. In 2020, the National Youth Tobacco Survey (NYTS) reported that 19.6% of high school students and 4.7% of middle school students are current e-cigarette users (Wang et al., 2021). While e-cigarettes had often been marketed as a safer option, the long-term effects of vaping on oral health remained poorly understood (Electronic Cigarettes and Oral Health - R. Holliday, B.W. Chaffee, N.S. Jakubovics, R. Kist, P.M. Preshaw, 2021, n.d.). Conventional cigarette smoking had been extensively studied and had been linked to various oral health issues, including periodontal disease, tooth decay, and oral cancer (Pushalkar et al., 2020). In contrast, the relative novelty of vaping had resulted in limited clinical research, particularly in regions such as Bangladesh where data on e-cigarette users had remained scarce. The oral cavity was recognized as home to a diverse microbial community that played a crucial role in maintaining oral and systemic health. Disruptions to this delicate balance, known as microbial dysbiosis, had been shown to lead to oral diseases such as dental caries, gingivitis, and periodontitis (Ganesan et al., 2020). Oral microbiome alteration had long been associated with smoking, usually in favor of pathogenic bacteria leading to inflammation and destruction of tissues. E-cigarettes, despite the lack of combustion, continued to expose the population to a plethora of chemical compounds, such as nicotine, propylene glycol, glycerin, and flavoring compounds, which might have influenced the oral microbial profile (Chopyk et al., 2021). It had been demonstrated in studies that both smoking and vaping were capable of influencing salivary flow rate, pH levels, and the composition of the oral microbiome. Nicotine, a common constituent of both cigarettes and e-cigarettes, had been recognized as a vasoconstrictor. This vasoconstriction was understood to reduce blood flow to the gingival tissues and to impair healing, thereby creating conditions that were favorable for bacterial proliferation (Wu et al., 2016). Additionally, the heating of e-liquid during vaping was found to generate harmful aldehydes, which further disrupted the balance of the oral ecosystem (Cichońska et al., 2022). It had been suggested by research that individuals who used both cigarettes and e-cigarettes were at greater risk of developing oral health complications. This elevated risk was attributed to the combined effects of combustion

products and vaporized chemicals, which were thought to exacerbate microbial imbalance more than either behavior alone. Elevated levels of pathogenic microorganisms such as *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Candida albicans* were frequently observed in such cases, leading to increased plaque accumulation, dental caries, and periodontal disease (Yang et al., 2023).

Despite the global focus on rising vaping rates, limited attention had been given to its effects on the oral microbiota within South Asian populations. This gap was particularly significant for Bangladesh, where tobacco use is prevalent and e-cigarette consumption has been increasing. Local research is essential for elucidating the specific microbial alterations in this population, as such changes were likely to vary according to genetic, dietary, and environmental factors (Chattopadhyay et al., 2024).

To fill this gap, this study aimed to compare the oral microbiome across four groups: non-smokers (NS), conventional cigarette smokers (CCS), e-cigarette users (ECS), and CCS+ECS users. Samples were taken from individuals aged 18 to 30 years in Dhaka, Bangladesh, including participants from BRAC University and nearby areas. Both males and females were recruited. To limit other influences, strict inclusion criteria were applied. Participants with serious dental problems, recent antibiotic use, dental braces, ongoing medication, or recent food intake were excluded. Only individuals who had consumed water before sample collection were included to maintain consistent oral conditions.

Bacteriological culture techniques and Gram staining had been used to detect bacterial population, and an emphasis was placed mainly on the proportion of Gram-positive and Gram-negative bacteria.

The outcome of this research had been deemed to bear significant public health policy implications. By enlightening the researchers about the manner in which various forms of smoking affected the oral microbial entities, this research had provided significant inputs which might inform oral interventions and public health policies accordingly. In addition, it had brought into sharp focus the importance of higher awareness of the risks of e-cigarette and conventional cigarette use, itself throwing into question the idea of the ECS as an absolutely safe alternative to the conventional combustible cigarette. Recognition of these dynamics had

been considered necessary in the development of focused strategies designed to avert oral disease in high prevalence areas of use of both conventional and electronic cigarettes.

1.2 Definition of Vaping

The term ‘vaping’ was characterized as inhalation and exhalation of aerosol created by an e-cigarette or a comparable device (Government of Canada, n.d.). The term “vape” was derived from “vaporize” or “vaporizer.” These devices were designed to generate an aerosol by heating a liquid, commonly referred to as e-liquid or vape juice, which was subsequently inhaled by the user (CDC, 2025).

Table 1.1: Types of E-Cigarette Devices

Types	**Definitions**	**Amount of nicotine**
Vape pens	These have a longer battery life and are marginally bigger than e-cigarettes. Usually cylindrical in shape, they are supplied with either a disposable cartridge or a refilling tank. (Mistle Liquid, n.d.)	0 mg/mL to over 50 mg/mL

Pod Devices	Pre-filled or refillable pods containing e-liquid with nicotine concentrations typically ranging from 20 mg/mL to 50 mg/mL (although lower and higher concentrations are also available) are frequently included with pod modifications. (Mistle Liquid, n.d.)	20 mg/mL to 50 mg/mL
Box Mods	These are more sophisticated, larger vaping devices with temperature and wattage controllable settings. They may hold tanks of different sizes and are often designed in the form of a box. (Mistle Liquid, n.d.)	0 mg/mL to 12mg/mL
Mechanical Mods	These are basic, uncontrolled gadgets devoid of any electronic parts. They are mostly utilized by experienced users who want complete control over their vaping experience. They are made up of a metal tube or box with a firing button. (Mistle Liquid, n.d.)	0 mg/mL to 12mg/mL

Disposable	These are one-time-use gadgets with a rechargeable or non-rechargeable battery and e-liquid pre-filled. If you don't want to deal with the trouble of constantly recharging or refilling your device, these are convenient. (Mistle Liquid, n.d.)	20 mg/mL to 50 mg/mL

1.3 Risks associated with smoking e-cigarettes

The utilization of e-cigarettes and vape devices was associated with several risks related to oral health complications, particularly in the development and exacerbation of dental plaque and oral biofilm. An understanding of these risk factors is considered essential for informing public health strategies and interventions aimed at mitigating the adverse effects of vaping on oral health.

1.3.1 Nicotine Exposure

E-cigarettes were typically found to contain nicotine, a highly addictive substance characterized by well-documented vasoconstrictive properties. Nicotine exposure has been linked to impaired gingival blood flow, delayed wound healing, and increased susceptibility to periodontal diseases (Jamal et al., 2018). Prolonged nicotine use was also understood to disrupt the balance of the oral microbiota, thereby promoting the proliferation of pathogenic bacteria within dental plaque (Benowitz, 2010). According to study, various factors, including an individual's weight, tolerance, and mode of intake (e.g., vaping, smoking, or swallowing), might affect the fatal dose of nicotine. Nonetheless, it was widely acknowledged that the lethal oral dose of nicotine for an adult typically ranged between 30 and 60 milligrams (mg). This indicated that an adult of average stature could have died from ingesting such an amount of nicotine at one time.

1.3.2 Alterations in Oral Microbiota

Vaping has been linked to alterations in the composition and quantity of oral microbial populations. The aerosolized ingredients of e-cigarettes may stimulate the growth and adherence of harmful bacteria, such as *Streptococcus* spp. and *Staphylococcus aureus*, to dental

plaque (Catala-Valentin et al., 2022). E-cigarette usage has been linked to changes in the composition and diversity of oral microbial populations. Individuals who use e-cigarettes have shown shifts in the number of *Streptococcus* and *Staphylococcus* species such as *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus pyogenes*, and *Staphylococcus aureus*. (S.S. Socransky, A.D. Haffajee, Cugini, Smith, & Kent, 1998). These modifications may lead to tooth plaque dysbiosis, which promotes the spread of cariogenic and periodontal infections. A microbiological in vitro study by R Holliday et all showed the potential mechanisms of the e-cigarettes impact on oral health. The study showed that different bioactive agents (Figure: 1) like glycerol or polyethylene glycol (PEG) carrying e-cigarettes might be the factors behind the microbiome composition alterations by putting harmful effects on periodontal fibroblasts and keratinocytes of the mouth. (Holliday et al., 2021).

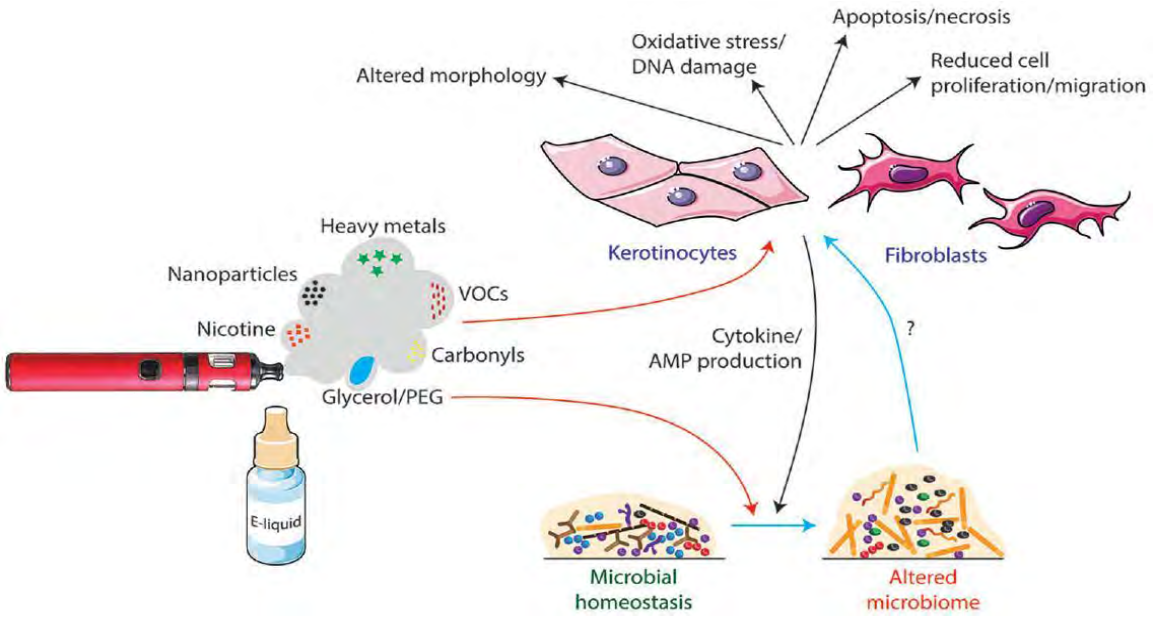

Figure 1.1: The potential pathways of the impact of e-cigarettes on oral health.

1.3.3 Dry mouth (Xerostomia)

E-cigarette aerosols desiccate the oral mucosa, causing users to experience dry mouth (xerostomia). Reduced salivary flow affects the removal of food debris and bacterial leftovers from the oral cavity, making people more likely to develop plaque and dental caries. Furthermore, xerostomia reduces the buffering ability of saliva, making teeth more vulnerable to demineralization by acid-producing bacteria. (Li, Lin, Xia, & Zhu, 2020).

1.3.4 Behavioral factors

E-cigarette users might have different oral hygiene and food habits, which contributed to the buildup of dental plaque and biofilm. The assumption that e-cigarettes could pose less danger than conventional tobacco products may cause users to underestimate the necessity of oral health maintenance, like regular dental inspections, brushing, and flossing. Furthermore, the simplicity and discreteness of e-cigarettes may encourage long-term and regular use, increasing oral health hazards (Panariello et al., 2025).

1.3.5 Pre-existing oral conditions

When people who already had oral health issues like dental caries or periodontal disease start using e-cigarettes, their symptoms might get worse. E-cigarette aerosols can worsen existing inflammation in periodontal tissues, jeopardize the integrity of dental restorations, and slow the healing of oral lesions (Khosravi et al., 2018). As a result, vaping might increase the risk of oral health issues for those who already had poor dental health.

1.3.6 Aldehyde production

Sumit Gaur and Rupali Agnihotri found some factors which were evident from research that sugar flavoured liquids of e-cigarettes were responsible for dental caries. Dental caries was promoted through various mechanisms by the aerosol generated from the e-cigarettes which were inhaled (Gaur & Agnihotri, 2023). Several studies have shown that hazardous aldehydes are formed in e-cigarette fumes during vaping. So far, aldehyde production has been attributed to the heat degradation of the primary components of e-cigarette e-liquids (propylene glycol and glycerol), with flavoring ingredients being neglected. Researchers assessed hazardous aldehydes produced by three popular e-cigarette brands using flavored and unflavored e-liquids. Thermal breakdown of flavoring chemicals dominates aldehyde production during vaping (Figure: 2), resulting in levels that exceed occupational safety regulations. Production of aldehydes was discovered to be exponentially dependent on the concentration of flavoring chemicals (Khlystov & Samburova, 2016).

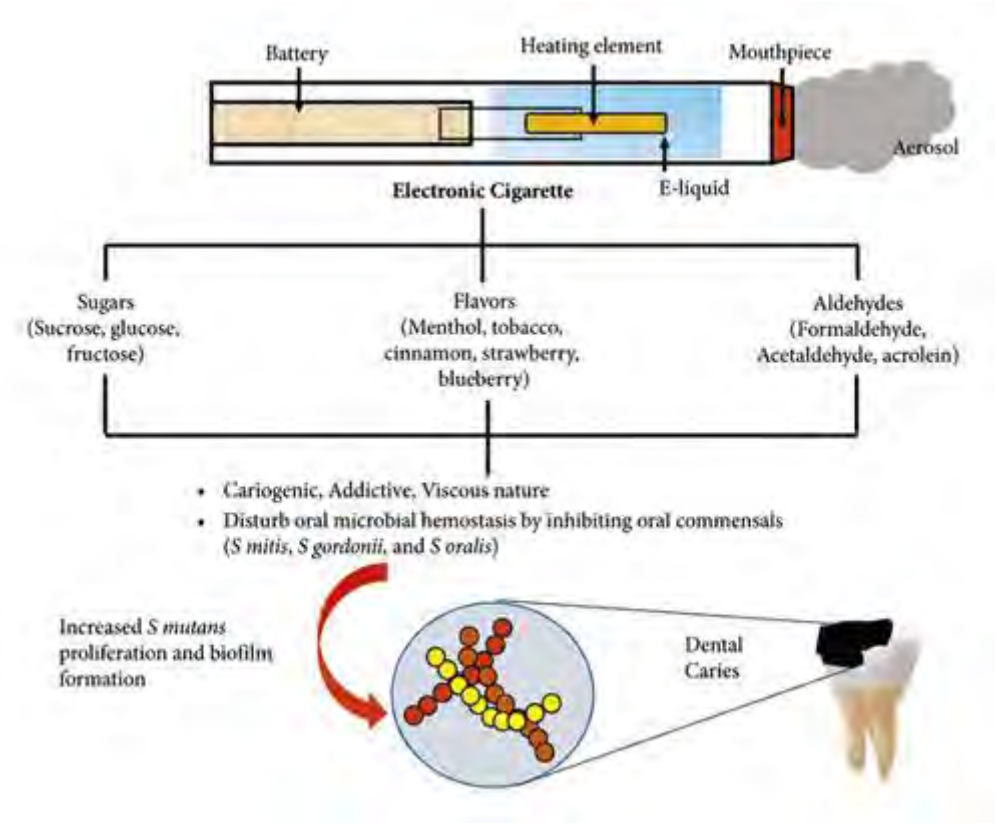

Figure 1.2: Factors involved in the effects of e-cigarettes.

1.4 Causes of Plaque

1.4.1 Nicotine Exposure

Nicotine is a common part of e-cigarette aerosols. It has been shown to affect the growth and harmfulness of oral bacteria. *Streptococcus* species, including *S. mutans*, are known to have receptors that bind to nicotine. These receptors may help the bacteria colonize and grow when nicotine is present (Huang et al., 2012). Besides, adherence to oral epithelial cells was increased by *Staphylococcus aureus* after getting exposed to nicotine.

1.4.2 Chemical Constituents

The aerosol from an e-cigarette had chemical ingredients like propylene glycol, glycerol, and flavoring agents, which were known to alter the pH and biochemical environment of the oral cavity. Such alterations may promote the proliferation of acidogenic and acid-tolerant bacteria, like *Streptococcus mutans*, which were responsible for the formation of plaque and tooth caries (Beauval et al., 2017).

1.4.3 Impaired Salivary Function

Since e-cigarette aerosols dry the oral mucosa, vaping had been associated with dry mouth (xerostomia). Lower salivary flow decreases the elimination of oral cavity food debris and bacteria, leading to the development of plaque and microbial colonization (Holliday et al., 2019).

1.4.4 Inflammatory Responses

Inhalation of e-cigarette aerosols may induce inflammatory reactions in the oral mucosa, which enabled pathogenic microorganisms to adhere and penetrate (Vardavas et al., 2012). *Streptococcus* spp. and *Staphylococcus aureus* possess virulence traits which they employ to evade the host's immune response and develop chronic infections in dental plaque.

1.5 Safety from cross-contamination

In order to acquire authentic and effective results, good laboratory practices were applied. Lab coats and hand gloves were worn during the experiment to avoid contamination. UV light was applied before using the laminar flow. Prepared culture media were put into a separate fridge at 4°C to avoid contamination. Sheep blood carrying conical flasks were wrapped with foil to avoid cross contamination and stored in the fridge at -20°C. Paraffin oil, 70% glycerol and 0.9% Saline were autoclaved before use. Each test tube, conical flask, glass vials and petri dish were washed before and after using it for experiment purposes. Petri dishes and glass vials were sterilized before applying culture media on them. Masking tape was wrapped over the stack of culture plates and paraffin paper was wrapped over the glass vial caps to avoid contamination. Also, Pipette tubes were autoclaved before using it for the experiment purposes. At the time of using pipette tubes, it was made sure that they were used inside the laminar flow. Cultured plates were kept in the culture fridge. Glass slides were handled wearing gloves during the gram staining experiment. Glass slides were washed at a safe distance from the staining pot. Each carrier of samples, saline, stock vials, media containing conical flasks and media plates were labeled with preparation date and name of the containing reagent.

1.6 Hypothesis of the study

The main hypothesis of this study was that the oral bacterial load and diversity would vary between conventional cigarette smokers, E-cigarette smokers and uses who smoke both conventional cigarette and E-cigarette.

1.7 Aim and objective of the study

The main aim of this study was to compare the load and diversity of oral bacteria between conventional cigarette smokers, E-cigarette smokers and uses who smoke both conventional cigarette and E-cigarette.

The specific objectives included:

1. To collect oral samples from study participants
2. To isolate bacteria from the collected samples
3. To estimate the bacterial load present in the collected samples.
4. To categorize the isolated bacteria based on Gram's staining and microscopic observations.

Chapter 2

2. Methods and materials:

This is case-control study in which oral bacteriological analysis was conducted on smokers and non-smokers.

2.1 Materials

2.1.1 Media and Reagents:

3. Table 2.1: Media and their compositions**.**

Category	**Blood Agar**	**Nutrient Agar**
Primary Nutrients	Tryptone, Peptone, Yeast extract (HiMedia Laboratories Pvt. Ltd., 2024)	Peptone, HM Peptone B / extract, Yeast extract (HiMedia Laboratories Pvt. Ltd., 2024)
Selective Agents	None (non-selective)	None (non-selective)
Differential Agents	5–10% sheep blood (for hemolysis observation) (HiMedia Laboratories Pvt. Ltd., 2024)	None
Buffering Agents	Sodium chloride (osmotic balance, minimal buffering from ingredients) (HiMedia Laboratories Pvt. Ltd., 2024)	Sodium chloride (osmotic balance, limited buffering) (HiMedia Laboratories Pvt. Ltd., 2024)
Solidifying Agent	Agar	Agar
Purpose	Supports fastidious organisms; identifies hemolytic patterns	General-purpose medium for non-fastidious bacteria
Use	40 grams/liter (HiMedia Laboratories Pvt. Ltd., 2024)	28 grams/liter (HiMedia Laboratories Pvt. Ltd., 2024)

2.1.2 Equipment

In this project, several pieces of equipment had been used. All of them are mentioned below:

Table 2.2: Equipment used in this experiment.

Equipment
Conical Flask
Glass Vial
Inoculating Loop
Glass Spreader
Glass Stirrer
Spatula
Paraffin Paper
Pipette (100 µl)
Pipette (1000 µl)
Pipette Tips
Tube Holder
Tissue Paper
Aluminum Foil
Hand Gloves (Box)
Masking Tape

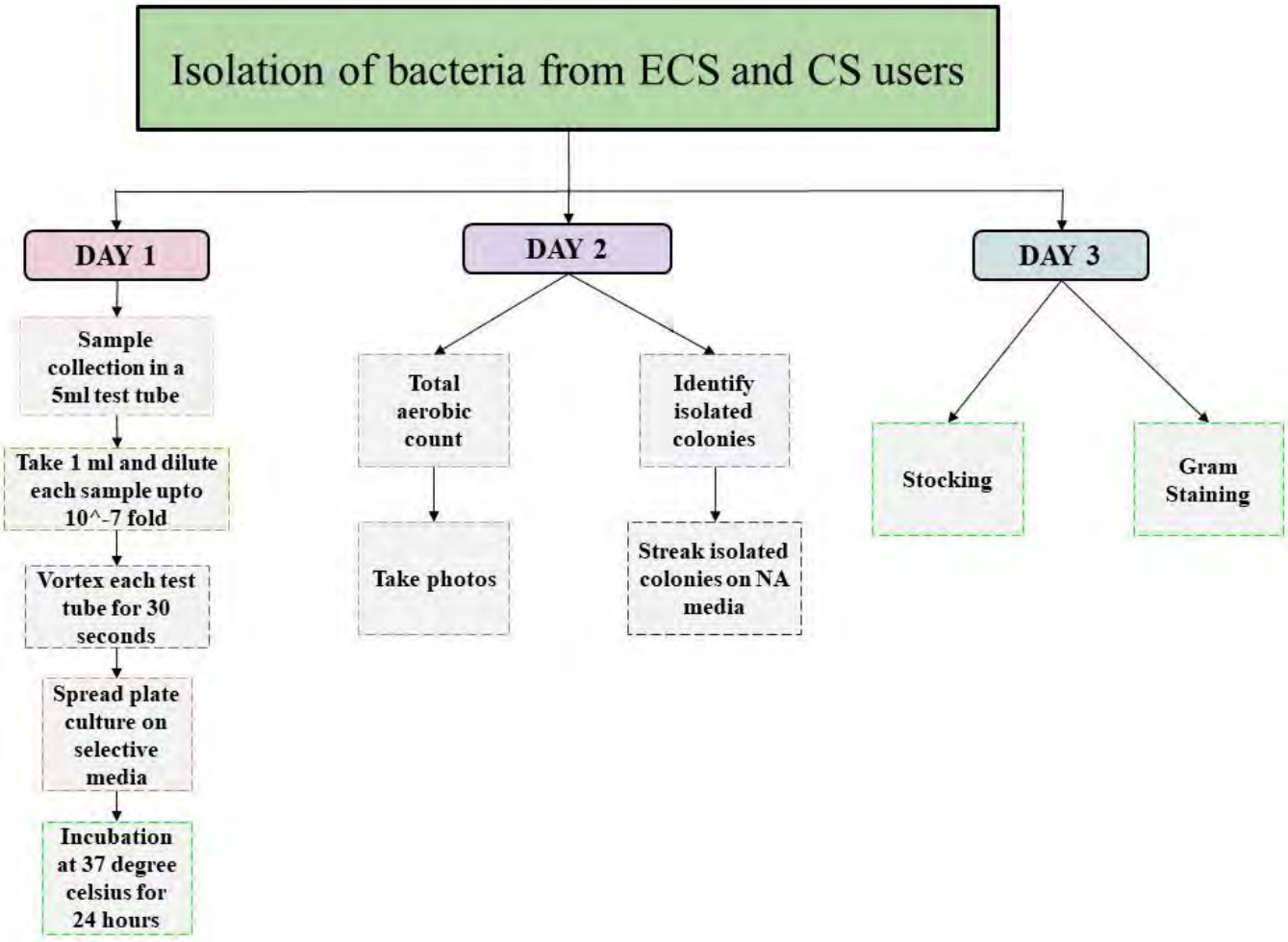

Figure 2.1: Workflow of the procedures.

2.2 Study participants

The study was conducted from April 2024 to July 2025. Forty study participants were recruited from BRAC University and divided in equal numbers (n=10) into 4 groups: i. Non-smokers, ii. e-cigarette users, iii. conventional cigarette users, and iv. users of both conventional and e-cigarettes

The following inclusion and exclusion criteria of study participants were included in the study:

Inclusion criteria:

- **i.** Participants must be between 18 to 30 years
- **ii.** Participants can be of any gender.

Exclusion criteria:

- **i.** Participants must not be on any type medications, especially antibiotics.

ii. Participants must not have braces on their teeth.
iii. Participants must not consume any solid or liquid food for at least 6 hours before sample collection.
iv. Participants must not brush their teeth, or chew gum for at least 6 hours before sample collection.

2.3 Collection of data

After obtaining informed consent, subjects were asked to provide their demographic and health-related information via a questionnaire.

2.4 Sample collection

Samples were taken from the subgingival areas of teeth. Every sample was collected with sterile cotton swabs and given the appropriate labels into the carrying containers of 0.9% saline. To avoid getting any of the samples contaminated, proper cautions were used at every stage: hands were sanitized using 70% ethanol solution and hand gloves were worn whenever the samples were being handled.

2.5 Sample processing

After collection, samples were diluted in 1 in 1o dilutions in 0.9% saline and processed for bacterial isolation and enumeration by standard plate methods.

2.5.1 Media Preparation and Procedure

To prepare each media, clean and washed conical flasks were taken. Clean foil paper was used to measure and cover the lead. A sterile stirrer was used for mixing the media well. Well measured distilled water on the measuring tube was taken before applying it with the media base.

To prepare the Nutrient Agar media, we used the HiMedia Nutrient Agar which took 28 grams to prepare 1 liter of media. We prepared them in 250ml conical flasks where it contained 7 grams of Nutrient Agar with 250ml distilled water. Every flask was labeled with name, creation date, and group name. Then each conical flask was autoclaved after covering the opening with foil paper. Each conical flask containing media was autoclaved once never twice before use.

To prepare Blood Agar media, blood was collected from a sheep and brought to the laboratory within 2 hours of collection. Blood collection was done carefully to keep contamination away. Blood was collected in glass beads containing conical flasks and were shaken after pouring blood into each flask so that the blood doesn't get clotted. The Agar Base was prepared by taking 40 grams of Blood Agar base for 1 liter. We had prepared media in 250ml conical flasks. In each 250ml flask, 10 grams of HiMedia Blood Agar Base was taken and mixed with 250ml of distilled water. Then they were labeled, covered with foil and autoclaved. Later 5% blood was mixed with the agar base and poured into plates for use. Blood was mixed into each Blood Agar base containing conical flask when it was under at least 50°C temperature.

2.5.2 Isolation and enumeration of bacterial isolates

The conventional spread plate method was employed as a standard microbiological technique for bacterial isolation and enumeration from diluted samples. The media were prepared and sterilized, then poured into sterile petri dishes and allowed to solidify. Serial dilutions of the sample, typically up to a 10^{-7} fold, were performed to achieve distinct and countable colonies. A fixed volume (0.1 mL) of the diluted sample was then transferred onto the agar surface using a sterile pipette and was spread uniformly with a sterile glass rod while the plate was gently rotated. The agar plates were incubated at 37°C for for 24 to 48 hours. After the incubation time, colonies were counted, and the microbial load of the original sample was calculated as colony forming units (CFU) per mL

2.5.3 Conventional Streak Plate Method

The streak plate method was used for isolating pure bacterial colonies from the culture plates. To begin, a sterile nutrient agar or other appropriate medium was poured into sterile Petri dishes, which were then allowed to solidify and slightly dry to eliminate excess surface moisture. An inoculating loop was sterilized by flaming it in a Bunsen burner until red-hot and subsequently allowed to cool to avoid killing the microorganisms. Once cooled, the sterile loop was dipped into the bacterial culture or sample to obtain a small amount of inoculum. The initial streak (primary inoculation) was conducted by carefully swiping the loop through one quadrant of the agar surface in a straight line or zigzag motion. The loop was re-sterilized to kill off any lingering organisms and cooled. The second streak was created by holding the loop through the border of the first streak and expanding outward into the second quadrant, thus diluting the bacterial population. The method of flaming, cooling, and streaking was reiterated for a third quadrant, and, if the three-quadrant method was used, a fourth, as needed. The loop

was finally sterilized after the last streaking procedure was completed. The prepared streaked plates were then inverted and incubated under conditions of 35–37°C for anywhere from 24 to 48 hours, contingent upon the organism. After incubation, isolated colonies were manifested in the areas of last streaking, demonstrating successful dilution and dispersal of individual bacterial cells (JoVE, n.d.).

2.6 Gram Staining

Gram staining was performed to identify bacteria as Gram positive or Gram negative. This method was used to distinguish bacteria according to differences in their cell wall composition. After the staining process, Gram-positive bacteria appeared purple or blue due to their thick peptidoglycan layer, while Gram-negative bacteria appeared red or pink, owing to their thinner peptidoglycan layer and higher lipid content.

For the staining procedure, a bacterial smear was prepared on a clean, sterile glass slide using a fresh culture. The smear was then heat-fixed and allowed to cool to room temperature. The primary stain, crystal violet, was applied to the smear and left for one minute. The slide was then gently and indirectly rinsed with distilled water until the runoff appeared clear. Next, Gram's iodine, serving as the mordant, was added and allowed to react for one minute before being similarly washed off. Following this, 95% ethanol, used as the decolorizing agent, was applied for 3 to 4 seconds and immediately rinsed off with distilled water to prevent over-decolorization. Subsequently, the counterstain, safranin, was applied for 30 seconds and then gently rinsed with distilled water. The slide was air-dried and then examined under a microscope using immersion oil. Upon microscopic examination, both Gram-positive and Gram-negative bacteria were identified based on their coloration (Smith et al., 2005). Bacteria were also categorized into rod-shaped and cocci-shaped based on their microscopic appearance. Observations were recorded and documented in a prepared data sheet.

Chapter 3

3. Results

Samples were collected from 40 individuals and they were categorized into 4 groups; non-smokers (NS), conventional cigarette smokers (CCS), E-cigarette smokers (ECS), and individuals who used both conventional cigarette smokers, E-cigarette smokers (CCS + ECS).

3.1 Growth on Nutrient Agar (NA) medium

Nutrient agar (NA) medium is a universal medium that allows the growth of all kinds of bacterial organisms and is used for total aerobic plate count. The following Figure (Figure 3.1) shows growth of bacteria on NA medium.

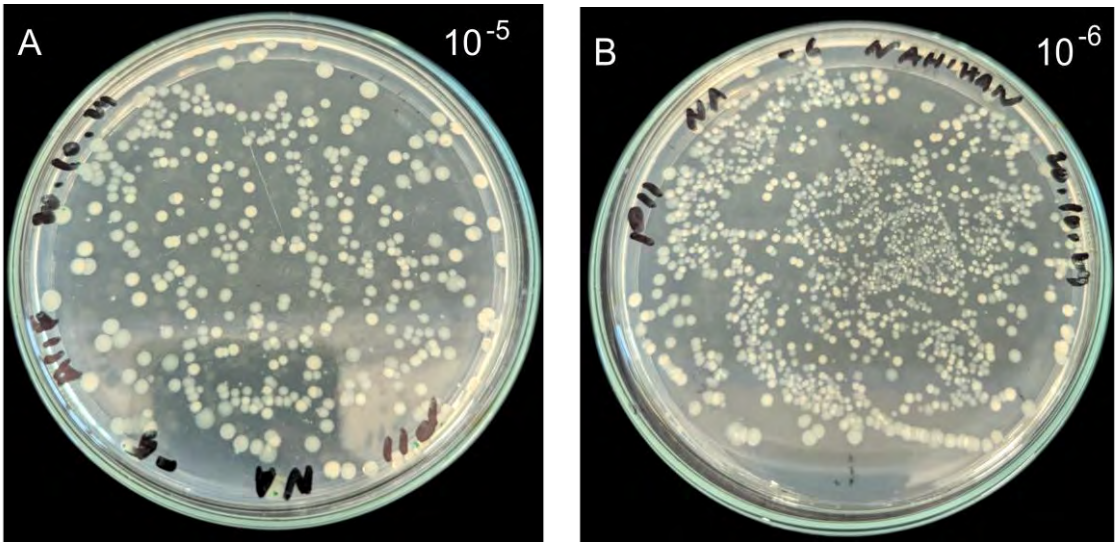

Figure 3.1: Growth on Nutrient Agar madium; A. Dilution factor 10^{-5}; B. Dilution factor 10^{-6}.

Figure 3.2 represents the average number of colony-forming units per milliliter (CFU/mL) observed on NA medium for each categorized group: NS, CSS, ECS and CSS + ECS. All bacterial samples were collected and cultured using the conventional spread plate technique following serial dilutions, and incubated at 35–37°C for a period of 24–48 hours. The results were recorded after the incubation phase and were used to calculate the average bacterial load for each group.

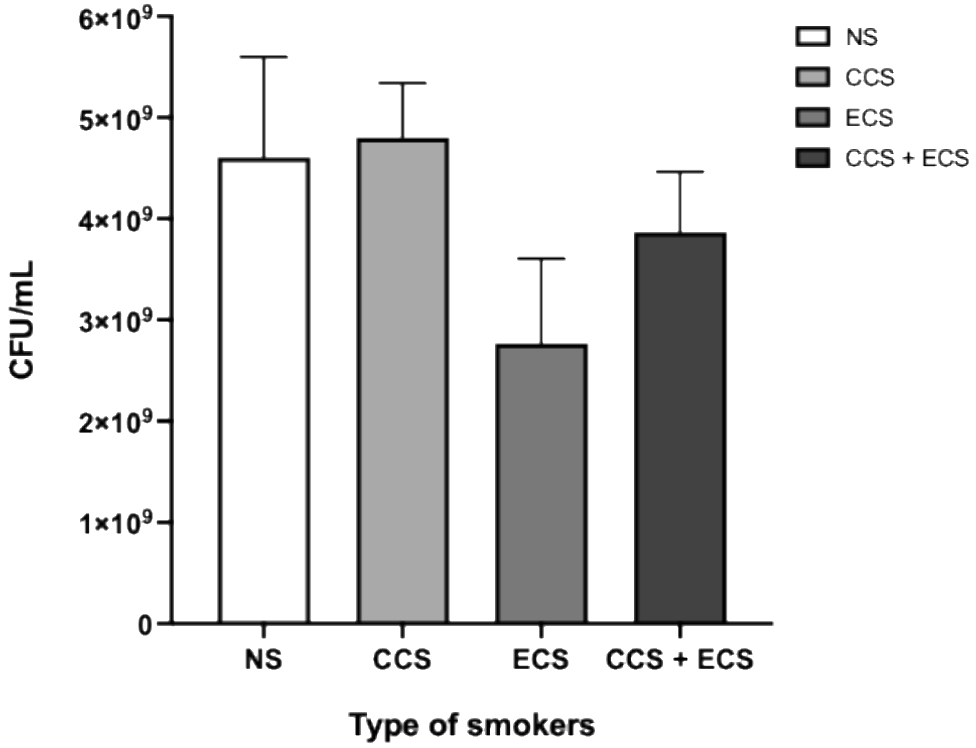

Figure 3.2: Average CFU/ml count on Nutrient Agar (NA) medium.

The results revealed a clear trend in bacterial load across the groups. The highest CFU/mL count were observed CCS group (4.8 × 10^9 CFU/ml), followed by NS group (4.6 × 10^9 CFU/ml), CCS + ECS group (3.9 × 10^9 CFU/ml) and ECS (2.8 × 10^9 CFU/ml). Although significant difference in bacterial load was not observed between any of the groups, these findings suggest that E-cigarette might have had an effect in lowering bacterial load.

3.2 Growth on Blood Agar Plates (BAP)

Blood Agar Plates (BAP) are regarded as an enriched and differential culture medium that is widely applied in microbiology for isolating and cultivating fastidious organisms, particularly those obtained from clinical samples. This medium enabled the growth of numerous bacterial species including fastidious bacteria and is especially valuable for observing bacterial hemolytic activity: alpha-hemolysis (partial lysis producing a greenish coloration), beta-hemolysis (complete clearing around the colonies), and gamma-hemolysis (absence of lysis). This feature makes blood agar an important medium for distinguishing bacterial species according to their hemolytic profiles, including *Streptococcus pyogenes* and *Streptococcus pneumoniae*.

Upon incubation, numerous colonies exhibited a wide range of sizes, shapes, elevations, margins, and surface features. Diverse pigmentation profiles from opaque white to light brown and light yellowish exhibited by these colonies indicated the presence of various bacterial genera. The hemolytic activity of the bacterial colonies was also observed since Blood Agar helps differentiate bacteria based on their ability to lyse red blood cells. Colonies with clear zones around their growth were classified as beta-hemolytic, showing complete hemolysis. Others displayed a greenish discoloration (alpha-hemolysis), indicating partial hemolysis, while some colonies did not change the surrounding medium, representing gamma-hemolysis or non-hemolytic activity. These phenotypic differences reflected the complex microbial ecosystem of the subgingival region (Figure 3.3).

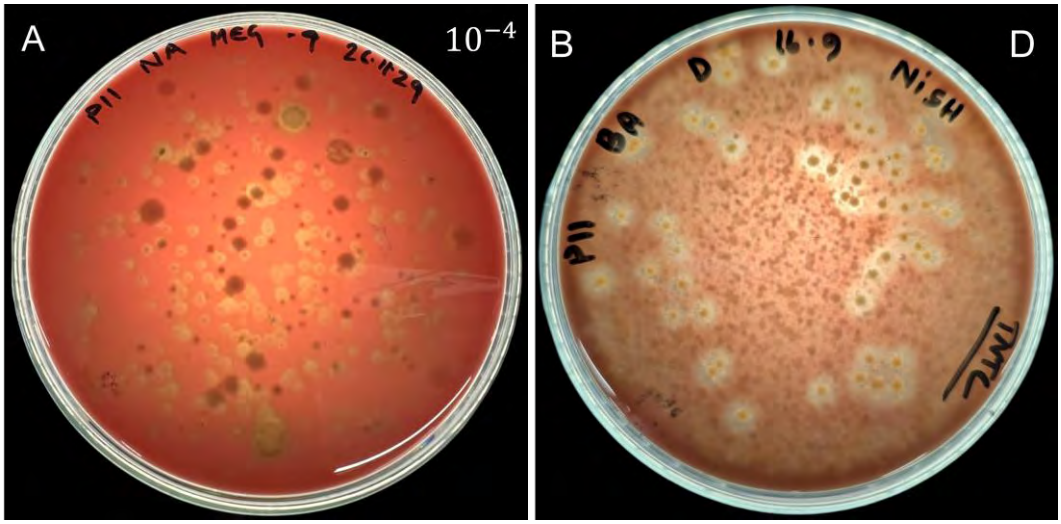

Figure 3.3: Bacterial growth on Blood Agar Plates (BAP).

In contrast to NA medium, BAP showed highest bacterial load from ECS group, with 1.5 x 10^7 CFU/ml (Figure 3.4). Additionally, CCS and ECS + CCS groups had comparable bacterial count (6.0 – 6.5 x 10^6 CFU/ml). The NS group had a bacterial load of 7.7 x 10^6 CFU/ml. A significant difference in bacterial load was observed between ECS group and CCS group (p = 0.02) and ECS group and CCS + ECS group (p = 0.02). Altogether, these findings indicate that Use of E-cigarette may have supported the growth of more fastidious bacteria in their users compared to other groups.

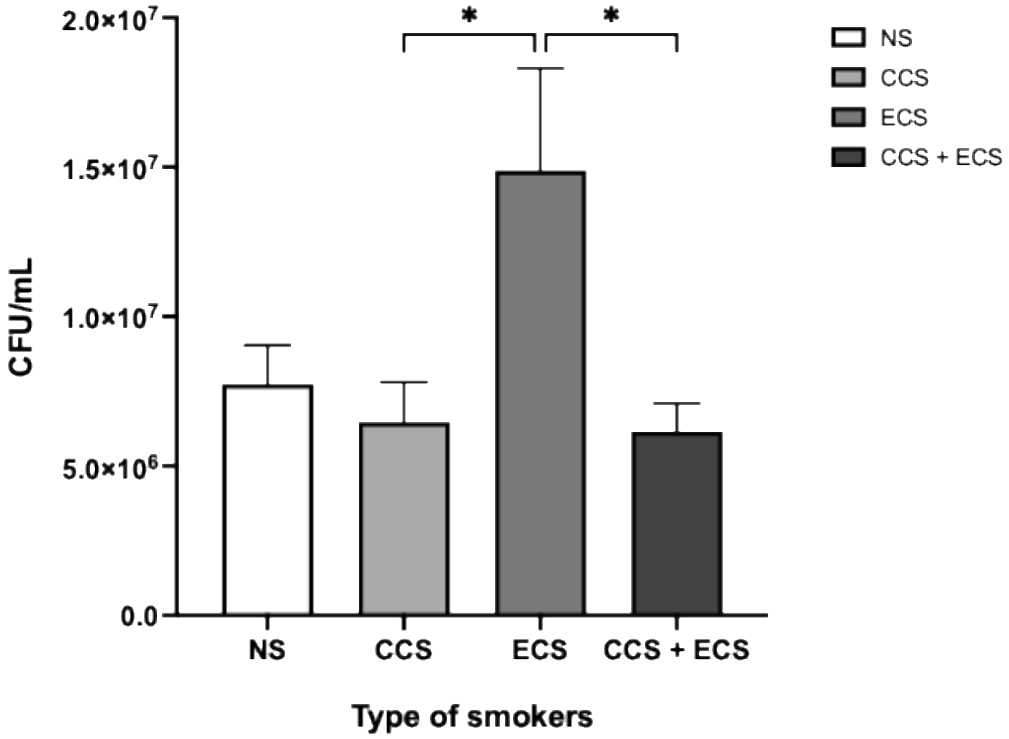

Figure 3.4: Average CFU/mL count on Blood Agar Plates (BAP).

3.3 Gram Staining

Both Gram-positive and Gram-negative bacteria were identified (Figure 3.5). All the bacterial isolates were categorised based on their staining data and shape such as Gram-positive rod, gram positive cocci; gram negative rod, gram negative cocci.

When the percentage of Gram-positive and Gram-negative bacteria was compared, it was observed that CCS group did not harbor any Gram-negative bacteria (Figure 3.6). In NS group, 58% bacteria were Gram-positive and 42% were Gram-negative. ECS group had the lowest percentage of Gram-negative bacteria (21%), while CCS + ECS group had equal proportion of Gram-positive and Gram-negative bacteria.

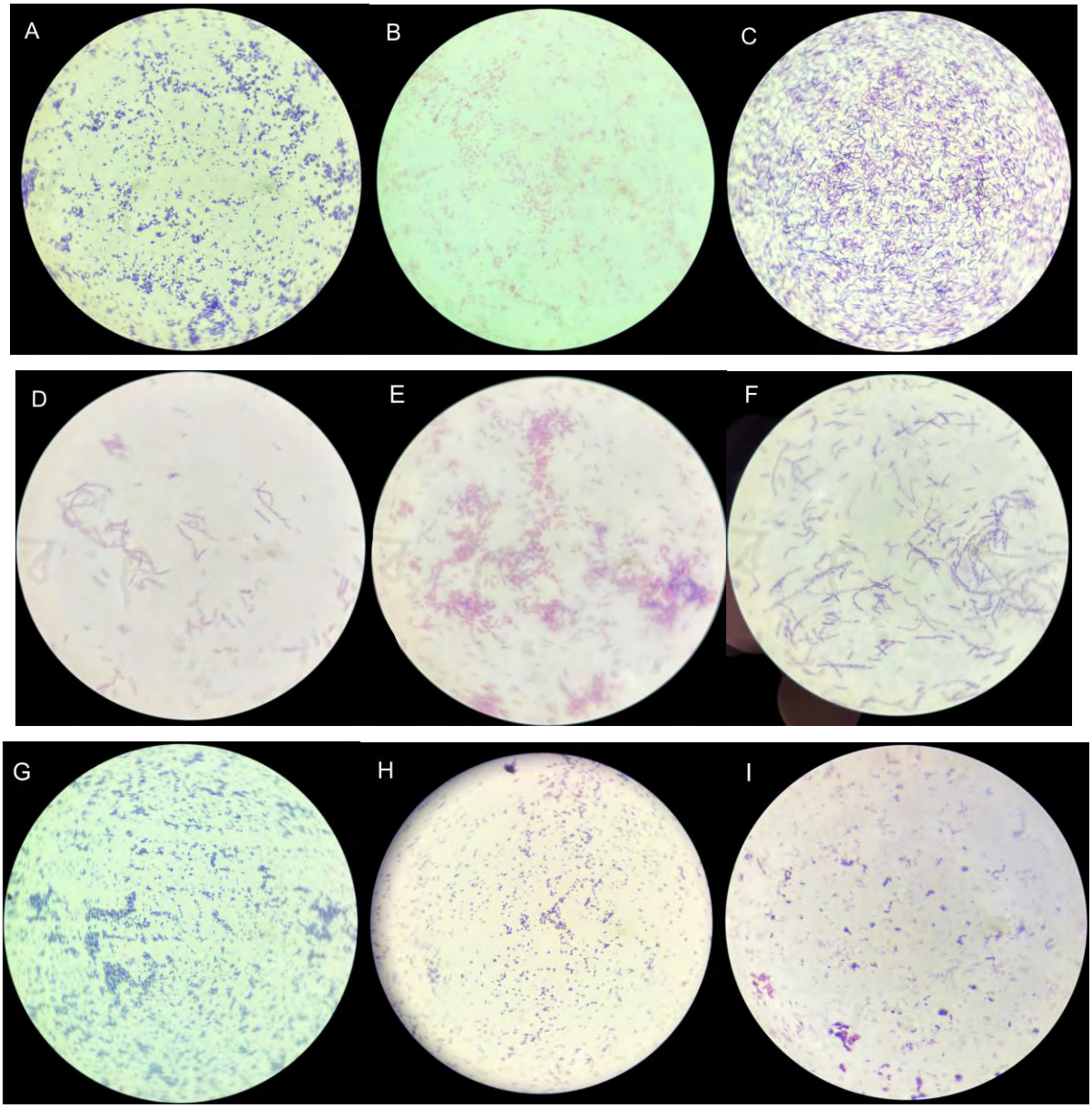

Figure 3.5: Gram staining results under microscope.

In the NS group, the percentage of Gram-positive rod-shaped bacteria was 16.67%, Gram-positive cocci-shaped bacteria was 41.67%, Gram-negative rod-shaped bacteria was 12.5%, and Gram-negative cocci-shaped bacteria was 29.17%. In the ECS group, the percentage of Gram-positive rod-shaped bacteria was 21.88%, Gram-positive cocci-shaped bacteria was 56.25%, Gram-negative rod-shaped bacteria was 15.63%, and Gram-negative cocci-shaped bacteria was 6.25%. In the CCS group the percentage of Gram-positive rod-shaped bacteria was 28.57% and Gram-positive cocci-shaped bacteria was 71.43%. In the CCS + ECS group, the percentage of Gram-positive rod-shaped bacteria was 50%, Gram-negative rod-shaped bacteria was 8.33%, and Gram-negative cocci-shaped bacteria was 41.66%. (Figure 3.7).

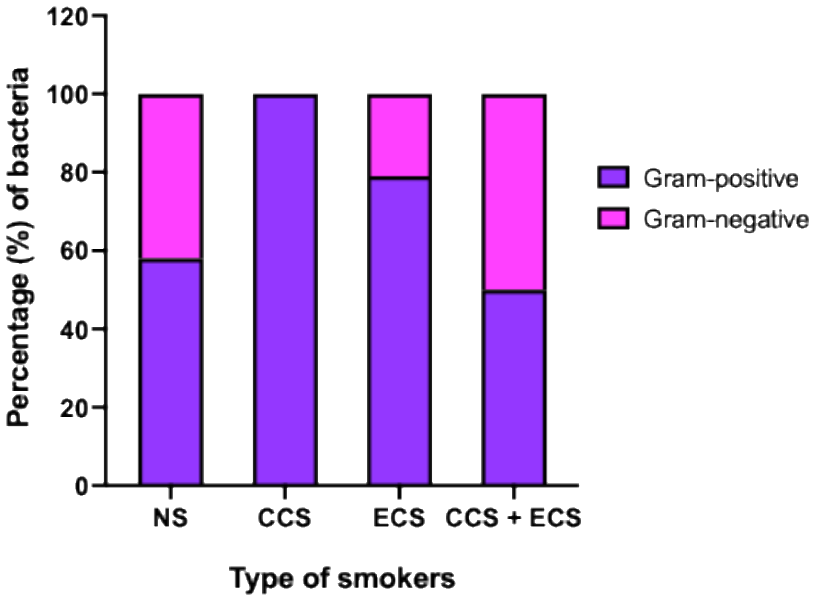

Figure 3.6: Proportion of Gram-positive and Gram-negative bacteria in different smoker groups.

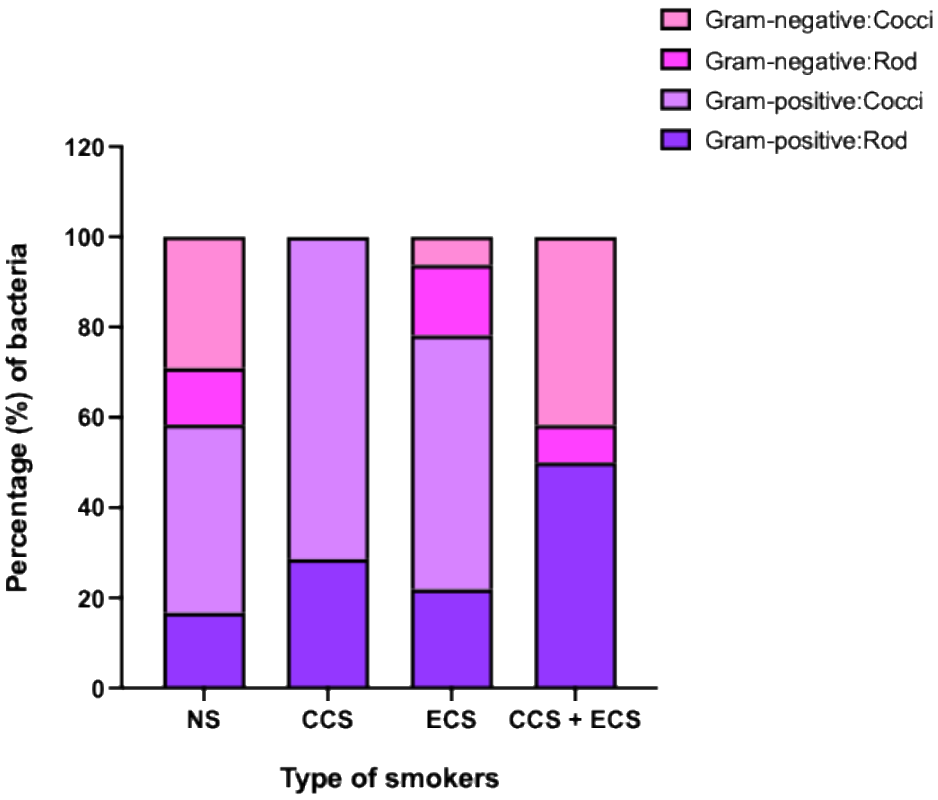

Figure 3.7: Percentage of both Gram-positive and Gram-negative rod-shaped and cocci-shaped bacteria in different smoker groups.

Chapter 4

4. Discussion

There are a few clinical studies where isolates from e-cigarette smokers were examined. This type of study had been done only a few times in Bangladesh though those research studies were conducted on isolated individuals. Currently, very little data is available on e-cigarette users and their clinical studies in Bangladesh. The purpose of this study was to conduct a comparative analysis of the oral microbiome across four groups: e-cigarette smoker (ECS), conventional cigarette smokers (CCS), CCS+ECS and non-smokers (NS). Oral swab samples were collected from each group and cultured, with Gram staining techniques used to identify and compare the presence of Gram-positive and Gram-negative bacterial species.

This study aimed to understand how ECS, CCS, and CCS+ECS usage affected the oral microbiome, with NS serving as the control group for comparison. It was hypothesized that ECS, CCS, and CCS+ECS users would show significant differences in their oral microbiomes when compared to NS. Also, it was hypothesized that pathogenic microorganisms such as *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Candida albicans* were present in greater abundance within the oral swabs of CCS and ECS, as the balance of the oral microbiota was disrupted by the chemicals and nicotine found in ECS as well as in CCS+ECS users. Furthermore, it was hypothesized that CCS+ECS users would present the largest microbial alterations, since the combined action of e-cigarette and conventional cigarette usage would likely propel an extensive impact upon the oral microbiome even further. Furthermore, it was hypothesized that Gram staining would identify a greater proportion of Gram-negative bacteria, especially in the ECS and CCS or in the both users, since these bacteria were also known to proliferate under decreased saliva flow and shifted pH conditions (Alsenani et al., 2025). Moreover, it was assumed that CCS+ECS users would present the largest microbial alterations, since the cumulative action of ECS and CCS usage would likely push an extensive impact upon the oral microbiome even further. Gram staining was assumed to examine these variations by visibly separating the Gram-negative and Gram-positive bacterial species of the oral specimens.

NA medium was utilized as an all-purpose medium to facilitate the growth of an extensive range of bacteria. Peptones, sodium chloride, and agar constituted the medium and supplied the requisite nutrients, maintained the osmotically balanced state, and solidified the medium,

respectively. Nutrient Agar was utilized mostly for the growth of Gram-negative and Gram-positive bacteria, encompassing species like *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* (Bauer et al., 2015). Because it was a non-selective medium, it contained no selective inhibitors or no specific indicators and, thus, allowed the growth of an extensive variety of microorganisms. The growth evident on Nutrient Agar was used to examine simple colony morphology and bacterial behavior under no selective impact and, therefore, was the best option when general bacterial growth was needed (Atlas, 2010).

BA was made to support the growth of fastidious bacteria and discriminate between bacterial species through their hemolytic capability. The medium was made up of peptones, sodium chloride, agar, and sheep blood, and the blood contributed extra growth factors integral for some bacteria. They were used to see the bacterial capability of digesting red blood cells, termed hemolysis, and were especially used to distinguish alpha-hemolytic, beta-hemolytic, and gamma-hemolytic bacteria. For instance, a greenish zone around the colonies was produced by partial hemolysis of *Streptococcus pneumoniae*, whereas a clear zone was produced by complete hemolysis of *Streptococcus pyogenes*. Gamma-hemolytic bacteria, like *Enterococcus faecalis*, did not cause any hemolysis (Macfaddin, 2011). BA was also used as an enriched medium for growing microorganisms that needed more complex nutrients, revealing the hemolytic patterns of various bacterial species (Macfaddin, 2011).

The NA media showed a lower bacterial count in NS and ECS compared to CCS and ECS, indicating a clear difference. In order to differentiate Gram-positive and Gram-negative bacteria, Gram staining was done to the isolated bacteria samples. Bacteria isolated from all four sample categories were stained and differentiated based on their Gram staining results. As most of the bacteria of the oral environment are typically Gram-positive. Our gram staining results showed that the percentage of the Gram-positive bacteria are higher than the Gram-negative bacteria. However, Gram-negative bacteria were also found but in lower amounts than the Gram-positive bacteria.

4.1 Key Findings of This Research

The oral microbiome of individuals who are CCS+ECS user was reported to be slightly altered, with an increased presence of Gram-negative bacteria noted in comparison with ECS and NS. In addition, suspected species such as *Streptococcus*, *Staphylococcus*, *Listeria*, and *Haemophilus* were found to be more prevalent in the oral cavities of CCS+ECS users, many of

which are associated with pathogenicity, biofilm formation, and inflammation. While the microbial composition and gram staining profiles of ECS and NS were found where Gram negative bacterial percentage were higher ECS than NS, the microbiota of CCS+ECS users was distinctly shifted toward a dominance of Gram-negative organisms. Although the net amount of Gram-positive and Gram-negative bacteria were found to be similar, the concentration of Gram-negative bacteria in CCS+ECS users was relatively higher when compared to ECS and NS.

These findings were examined by Gram staining methods. We confirmed that exposure to CCS+ECS created a unique and unbalanced oral environment. This environment favored the growth of Gram-negative organisms, some of which could be harmful. Researchers believed this microbial imbalance raised the risk of oral diseases. It also reflected the effects of using both tobacco and e-cigarettes on the oral bacterial community. These findings were also confirmed by gram staining techniques, verifying that CCS+ECS exposure to smoke and vapor had produced an imbalanced and distinctive oral environment conducive to Gram-negative organism growth, some of which could be pathogenic. The resultant microbial imbalance was assumed to raise the possibility of developing oral disease and reflected the effect of combined e-cigarette and combustible cigarette use on the oral bacterial community. The study demonstrated that participants who practiced electronic cigarettes and conventional cigarettes showed significant alterations of their oral microbiome, best characterized by increased Gram-negative bacterial species presence relative to the other users. In comparison to the exclusive use of either tobacco or e-cigarette, e-cigarette and conventional cigarette use was linked to greater oral microbial environment disruption.

These outcomes presented evidence that CCS+ECS use may present increased oral health risks and challenged the prevailing belief of electronic cigarettes being a safer option when used in conjunction with conventional smoking. A higher prevalence of Gram-positive bacteria was identified in the oral swabs of CCS through gram-staining when compared with the NS. The increased occurrence reflected the microbial changes previously linked to exposure from tobacco smoke. This study stated that the use of smoking conventional cigarettes affected the oral microbial environment by favoring the development of Gram-positive bacteria. In contrast, ECS exhibited an oblique proportion of Gram-positive bacteria compared to the NS or CCS. ECS and NS groups contained Gram-negative bacteria in the oral swabs, although the NS exhibited a slightly greater count of Gram-negative bacteria compared to the ECS group.

4.2 Unexpected Results

Oral microbiome composition of various user groups was examined to identify the influence of smoking, vaping, and use of both upon microbial community structure. Though significant differences existed in some bacterial populations, mainly the higher occurrence of Gram-positive bacteria in CCS+ECS, an unexpected outcome was overall bacterial load and microbial diversity maintained relatively consistent across CCS, ECS, CCS+ECS, and NS. Surprisingly, CCS group had the highest percentage of Gram-positive bacteria than the other groups where no Gram-negative bacteria was present. CCS+ECE group had the lowest percentage of Gram-negative bacteria compared with the other groups. However, CCS group showed a higher proportion of Gram-positive bacteria than other groups while CCS+ECS group represented equal distribution of Gram-positive and Gram-negative bacteria. Therefore, the interplay of these factors may mask or moderate the impact of tobacco and e-cigarette exposure upon microbial diversity. The result points towards microbial community composition and functional potential, and not just by diversity measures, were crucial items in the comprehension of the oral health outcomes of tobacco and e-cigarette usage.

Furthermore, an unexpected outcome was noticed while comparing the gram staining outcomes of CCS and NS. CCS were noticed having higher Gram-positive bacteria compared to NS when they consumed conventional cigarettes. The result came as a surprise because, according to previous research, it was identified previously that conventional as well as electronic cigarettes adversely influenced salivary biomarkers, namely salivary flow rate, pH, etc. (Zięba et al., 2024), which gave an impetus to the development of Gram-negative bacteria (Cichońska, Kusiak, Piechowicz, & Świetlik, 2021). However, in the present study, the rate of Gram-positive bacteria in CCS was found to be higher than in NS. Upon further investigation, we found that ECS and NS showed a variation of Gram-negative bacteria. Although NS were expected to have the lowest concentration of Gram-negative bacteria, this was not the case, which further suggested that factors beyond smoking and vaping behaviors, such as individual host genetics, diet, and oral hygiene practices, also strongly affected oral health.

4.3. Implications of our Findings

4.3.1 Impact of Smoking and Vaping on Oral Microbial Composition

The observation that CCS, in comparison with NS, exhibited a higher prevalence of Gram-positive bacteria was unexpected, since smoking had generally been associated with increased

Gram-negative organisms due to its influence on salivary flow, pH, and overall oral health. It was suggested that the inflammatory response caused by tobacco smoke may have favored Gram-positive bacteria, which are more resilient in such conditions. Additionally, Gram-negative bacteria were found to be somewhat more prevalent in e-cigarette users than in CCS, supporting previous studies that indicated e-cigarette vapor could have disrupted the oral microbiome and promoted Gram-negative organisms.

These results implied that smoking and vaping affected the oral microbiome in different ways. The microbial profiles of ECS and NS showed similar results due to factors like different lifestyle, oral hygiene which influenced the residual Gram-negative bacteria of the microbiome.

4.3.2 The Role of Host Factors in Oral Microbial Community Composition

This investigation pointed out that even though there were alterations in the oral microbiome of ECS, and CCS+ECS, microbial diversity and bacterial burden were maintained between groups. Stability here indicated the role of host factors such as genetics, oral health, diet, and environmental exposures in determining the oral microbiome, and potentially buffering the influence of vaping and smoking. The results indicated that looking only at the microbial diversity might not fully portray the impact of tobacco and e-cigarette use on the oral health of an individual. Instead, it was important to consider both the composition and the functional capabilities of the oral microbes. To gain clearer insight and prediction of the long-term effects of smoking and vaping on oral health, it was suggested that future research should be conducted with consideration of broader influences such as diet and genetics.

4.3.3 Implications for Public Health and Policy

These findings were considered a significant public health concern and were also regarded as highly relevant to the health effects of e-cigarettes. Although e-cigarettes had often been promoted as a safer substitute for conventional cigarettes, the study indicated that the combined use of both products might have been more detrimental than either habit alone, potentially leading to greater microbial imbalance within the oral cavity and a higher risk of oral diseases and other health complications. However, questions were raised by the study about the broader public perception that e-cigarettes were safer for smokers. As per the results, it showed, elevation of CCS+ECS use nowadays could complicate efforts to reduce smoking-related problems and improve overall health outcomes. It was recommended that public health campaigns focus on the risks of CCS+ECS use, and that policies be updated to reflect the

dangers of using both e-cigarettes and conventional cigarettes concurrently. This could lead to more comprehensive strategies aimed at reducing CCS+ECS use and its associated risks. In situations where saliva flow was decreased, such as in cases of dry mouth caused by vaping, an environment was created that could encourage the overgrowth of *Candida* species, leading to oral candidiasis (thrush). This condition was marked by white patches in the mouth and was known to contribute to the sensation of a dry mouth. *Streptococcus mutans,* one of the causative agents of caries (cavities), was found to flourish under conditions of dry mouth. When the saliva was not effective in neutralizing acids, *S. mutans* multiplied faster and presented an increased risk of developing tooth decay (Bauer et al., 2015). *Porphyromonas gingivalis*, the main pathogen of causing periodontal disease, was found to increase in the oral cavity concentration owing to the reduced saliva flow brought about by dry mouth. The bacteria gave rise to and contributed to the development of gingivitis and periodontitis, particularly among individuals who experienced dry mouth (Kistler et al., 2013). In addition, *Streptococcus sanguinis*, an oral cavity beneficial bacterium used to inhibit the growth of pathogenic bacteria like *S. mutans*, was also compromised by reduced salivary output from dry mouth. The consequence saw pathogenic species develop freely, increasing the risks of oral infection and other conditions (Zaura et al., 2014). From the above, the following conclusion was possible: because almost half of e-cigarette product users confessed to having experienced dry mouth, the possibility of an increase of pathogenic bacteria like *Streptococcus mutans, Porphyromonas gingivalis*, *Candida* species, and *Candida albicans* (fungus) was beyond question.

The study demonstrated that individuals who simultaneously used both conventional cigarettes and e-cigarettes showed notable alterations in their oral microbiome, particularly an increased presence of Gram-negative bacteria. Although the overall bacterial diversity appeared to remain consistent across CCS, ECS, and NS, this stability was likely influenced by additional factors such as genetics, diet, and oral hygiene. Interestingly, CCS were found to have a higher proportion of Gram-positive bacteria than NS, which was contrary to the hypothesis that a greater percentage of Gram-negative bacteria would be observed in this group. On the contrary, CCS and ECS showed less percentage of Gram-negative bacteria compared to those who use both. These results suggested that CCS+ECS usage may elevate oral health risks and raise concerns about the perceived safety of e-cigarettes when used alongside conventional tobacco products.

4.4 Comparative Analysis with Existing Literature

Our findings were found to indicate that CCS+ECS users showed a microbiome shift toward Gram-negative bacterial dominance, whereas CCS were observed to have an unexpectedly higher prevalence of Gram-positive bacteria in their oral samples. When comparisons were made with existing literature, both similarities and differences were identified. Many high-throughput sequencing studies had reported that cigarette smoking created an environment favoring Gram-negative anaerobes and a relative decline in Gram-positive bacteria, a shift often linked to periodontal disease progression (Chattopadhyay et al., 2024). For example, it was observed by Chattopadhyay et al. (2024) that CCS had an increased abundance of Firmicutes (a phylum of mostly Gram-positive bacteria) but a decreased presence of Proteobacteria (which includes many Gram-negative species) compared to NS (Chattopadhyay et al., 2024). This was seen to partially align with our observation of a different microbial profile in CCS, though our culture-based data specifically revealed a higher proportion of Gram-positive isolates in CCS. A culture-based investigation conducted by Khan et al. (2024) similarly reported that 67% of Gram-positive bacterial isolates in their study were collected from CCS (Khan et al., 2024). *Staphylococcus aureus* and *Streptococcus pneumoniae* were identified as prevalent Gram-positive species in CCS saliva (Khan et al., 2024), supporting our result that smoking could enrich certain Gram-positive organisms. This culture-based evidence was seen to echo our gram staining findings and suggested that some Gram-positive bacteria (such as *staphylococci* and *streptococci*) were resilient within the inflammatory, tobacco-exposed oral environment. In contrast, vaping was shown to be associated with slightly increased Gram-negative bacterial presence, which contradicted with our observation that ECS exhibited less Gram-negative counts than NS (Cichońska et al., 2021), reported that ECS users demonstrated a significantly greater frequency of Gram-negative rods (15.63% of subjects) compared to conventional cigarette smokers (CCS) where no Gram-negative rods were present (Cichońska et al., 2021). No significant difference in Gram-positive bacteria between these groups was found (Cichońska et al., 2021), implying that vaping uniquely promoted Gram-negative microbial colonization. Our results were observed to concur, as while the overall profiles of ECS and NS remained closely similar in terms of Gram-negative bacteria, vapers were found to harbor slightly more Gram-negatives. It was also noted by researchers that CCS+ECS harbored several known pathogenic microbes that were not as commonly seen in exclusive ECS or CCS (Catala-Valentin et al., 2023). Specifically, that study was reported to show that certain periodontal pathogens and opportunistic bacteria were uniquely abundant in CCS+ECS, suggesting that the combination of smoke and vapor exposures created a more

favorable niche for oral pathogens. Although detailed taxonomic data on CCS+ECS remained limited, our findings were seen to align with this emerging pattern. This phenomenon was believed to carry significant implications. CCS+ECS were thought to potentially face the worst of both exposures: the low-oxygen, nutrient-rich environment created by smoking that encouraged anaerobes, along with the selective pressures from vaping (such as altered saliva pH or the effects of propylene glycol) that favored different bacteria. Indeed, it was highlighted in one narrative review that conventional and e-cigarette use was common and may have caused oral health harms comparable to smoking alone (or possibly even worse), underscoring that using e-cigarettes in addition to conventional cigarettes did not mitigate the microbial or clinical risks of smoking (Holliday & Chaffee, 2021). Our microbiological evidence was found to strongly support this view. Pushalkar et al. (2020) noted that nearly three-quarters of CCS were affected by gum disease compared to 28% of NS, with ECS positioned in between at 43% (Pushalkar et al., 2020), which was observed to imply that CCS+ECS could trend toward the upper end of disease prevalence. Although direct studies on CCS+ECS, oral microbiomes were still emerging, our results were seen to parallel these early reports: e-cigarette and conventional cigarette use amplified microbial imbalances, marked by a convergence of smoking-related dysbiosis and vaping-related dysbiosis, leading to a more diverse array of potential pathogens. This comparative outcome was believed to challenge the notion that adding e-cigarettes was harmless; instead, e-cigarette and conventional cigarette users were not observed to retain the healthier microbial profile of NS or ECS, but rather displayed a microbiome indicative of compounded risk. Our work and the existing literature were both observed to highlight that e-cigarette and conventional cigarette use likely posed an elevated threat to oral health, as evidenced by the microbial shifts toward pathogenic species. A striking aspect of our study was found to be that despite clear shifts in specific bacterial groups, the overall bacterial load and CCS, ECS, CCS+ECS and NS all showed comparatively constant alpha-diversity of the oral microbiome. In other words, it was shown that smoking and vaping altered which bacteria were present (and their proportions) more than the total number of species or richness/diversity metrics. This finding suggested that host and environmental factors might have helped buffer the community against complete diversity loss, even as community composition changed. When these observations were compared to previous studies, it was found that reports on diversity changes due to smoking/vaping were mixed, further indicating the influence of individual variability. Some studies were shown to agree with our observation of limited diversity changes: for instance, (Liu et al., 2025) reported no significant difference in saliva microbial alpha-diversity between ECS and NS, although CCS did exhibit altered diversity in

their cohort (Liu et al., 2025). This aligned with our finding that ECS and NS shared broadly similar diversity profiles, suggesting that vaping alone might not drastically reduce microbial variety in the short term. Importantly, our interpretation that factors beyond smoking/vaping (such as host genetics, diet, and hygiene) heavily influenced the oral microbiome was echoed by other researchers. It was widely agreed in the literature that interpersonal variation in oral microbiota was substantial (Chattopadhyay et al., 2024). Even under the stress of smoking, each individual's baseline microbiome, immune responses, and behaviors (such as oral hygiene practices, dietary habits, and frequency of dental care) were shown to shape how their bacterial communities shifted. For example, one study pointed out that oral microbiome research was difficult to compare across studies due to variability in sampling and host factors, and that while smoking was recognized as a significant factor, its impact could be modulated by these other influences (Chattopadhyay et al., 2024).

4.5 Limitations

Despite several noteworthy findings, this study faced a number of important limitations. The unavailability of other specialized media for isolating *Streptococcus mutans* constrained the scope of microbiological analysis, which might have enabled this research to make a broader contribution as one of the few clinical studies on oral health in the South Asian region. The study was influenced by the sample donors as it was challenging to find individuals who used e-cigarettes only and this narrowed the sample size. Consequently, no significant quantitative data were collected as ECS were found rarely. Additionally, the scarcity of study-based supplementary materials on e-cigarettes restricted a deeper academic exploration of this emerging topic. The study also did not consider the potential effects of different vape device types on the oral microbiome, nor did it examine the contributions of e-cigarette use to viral and fungal communities in the oral cavity. Furthermore, the research did not include an assessment of key salivary biomarkers such as saliva flow rate, pH, oxidative stress markers, and inflammatory cytokines, which could have provided valuable context regarding how microbial shifts correlate with physiological changes in ECS and CCS. Together, these limitations highlighted areas for future research to build on and deepen the knowledge of how e-cigarettes affect oral health.

4.6 Suggestions for Future Research

1. Impact of vape flavors and e-liquid composition on oral microbiota.
2. Molecular identification of stock cultures and compare it to the standard oral flora species.
3. Role of nicotine concentration in vaping-induced microbial dysbiosis.
4. Host Immune Responses to Vaping-Associated Microbiome Shifts.
5. Comparison of oral microbiome recovery after vaping cessation.
6. Molecular and Metagenomic Profiling of Oral Microbial Communities of long term vape users.
7. Effect of devices which were used for respiratory muscle training.

Chapter 5

5. Conclusion

This study had been undertaken to examine the effects of different smoking behaviors as such conventional cigarette smoking (CCS), e-cigarette smoker (ECS) use, CCS+ECS usage, and NS on the oral microbiome, using culture-based analysis and Gram staining methods. The aim had been to address a notable gap in localized research on oral microbial shifts, particularly in the context of rising vaping trends in Bangladesh.

Key findings indicated that CCS+ECS users had exhibited a distinct microbial profile, with a marked increase in Gram-negative bacterial presence. Unexpectedly, CCS had shown a higher proportion of Gram-positive bacteria in contrast to CCS+ECS, while ECS had demonstrated a microbial pattern of Gram-negative bacteria lower. However, it closely resembled that of NS but with slightly elevated Gram-negative counts.

These findings were valuable in explaining the smoking habit causing microbial changes. It suggested that the use of e-cigarette and conventional cigarette were dangerous for their users. Oral health problems as they experienced combined effects of vapor and smoke resulting in oral microbial disbalance. Also, it supported that e-cigarette and conventional cigarette use could threaten oral health compared to the habit of smoking and vaping only.

From public health standings, these were highly valuable insights. These findings could be a great help by shaping clinical practices and aware public where e-cigarette usage along with the high tobacco consumption had risen in our country. The observed microbial shifts provided baseline data that could help shape oral health interventions, risk communication, and tobacco control policies.

While this study made some contributions in this context, it had some limitations as well. First, it depended on the culture-based methods and gram staining which were effective for the pattern identification of the general microorganisms. Though, it missed identification through molecular techniques. Second, the sample size, age range (18-30 years), and recruitment from a single urban area (Dhaka) might have limited the generalizability of the findings. Additionally, individual participants' oral hygiene routines and dietary habits had not been controlled beyond basic screening, which could have influenced the results.

Future research had been recommended to expand on these findings by incorporating high-throughput sequencing technologies for deeper taxonomic resolution and functional profiling of oral bacteria. Longitudinal studies should be conducted to observe microbial changes over time, especially as vaping behaviors evolve. Research could also explore the oral microbiome in specific populations, such as adolescents or individuals with pre-existing health conditions, and examine the effects of various e-liquid formulations and nicotine concentrations.

In conclusion, the results had emphasized the significance of comprehending the microbial consequences of e-cigarette use, particularly in combination with conventional smoking. The observed microbial imbalances underscored the need for ongoing vigilance in assessing the safety of emerging tobacco alternatives. It was hoped that this research would contribute to a broader dialogue on tobacco harm reduction, public health policy, and clinical awareness in regions where smoking patterns continued to shift.

Chapter 6

6. References

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