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Research Article

Evaluation of Pharyngeal Commensalism by Aerobic and Capnophilic Bacteria Among Healthy Individuals Residing in and Around Northern Bihar

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ABSTRACT

Background: The human pharynx harbors a diverse community of commensal microorganisms that maintain mucosal health and prevent pathogen overgrowth. Understanding the composition of normal pharyngeal flora is essential for distinguishing colonization from infection and guiding antimicrobial stewardship.
Objective: To evaluate the pattern and diversity of aerobic and capnophilic bacterial flora colonizing the pharynx of healthy individuals residing in and around Northern Bihar.
Materials and Methods: A cross-sectional study was conducted in the Department of Microbiology, Mata Gujri Memorial Medical College and L.S.K. Hospital, Kishanganj, Bihar, over 18 months (June 2023–December 2024). Throat swabs from 200 asymptomatic volunteers were cultured on Blood Agar (aerobic) and Chocolate Agar (capnophilic) using a candle-jar method. Isolates were identified up to the genus level by standard microbiological procedures. Data were analyzed using SPSS v26.0 with Chi-square/Fisher's exact tests; $p < 0.05$ was considered significant.
Results: Among 200 samples, 95% were positive for aerobic growth and 90% for capnophilic growth, with an overall combined culture positivity of 99%. *Viridans group Streptococcus* was the most prevalent organism (85%), followed by *Neisseria* spp. (55%), *Staphylococcus aureus* (25%), and *Moraxella catarrhalis* (20%). Mixed bacterial colonization was frequent, particularly between *Viridans Streptococcus* and *Neisseria* spp.
Conclusion: Healthy individuals in Northern Bihar harbor a rich pharyngeal microbiota dominated by *Viridans Streptococcus* and *Neisseria* spp. The detection of potential opportunistic pathogens like *S. aureus* highlights the clinical importance of monitoring commensal flora. Dual aerobic and capnophilic culture methods effectively capture this microbial diversity, providing valuable baseline data for infection surveillance and antibiotic policy formulation.

Keywords*: Pharyngeal flora, Aerobic bacteria, Capnophilic bacteria, Commensalism, Northern Bihar, Viridans Streptococcus, Neisseria spp.*

INTRODUCTION:

The human pharynx serves as a critical ecological niche for a diverse array of commensal and pathogenic microorganisms, playing a pivotal role in respiratory health and disease prevention.[1] Commensal bacteria in the pharynx contribute to colonization resistance, limiting the overgrowth of opportunistic pathogens through competitive exclusion and modulation of local immune responses.[2] Among these, aerobic and capnophilic bacteria—such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria* species—are of particular interest due to their dual role as both symbiotic colonizers and potential pathogens responsible for respiratory infections, meningitis, and otitis media.[3,4] Understanding the baseline prevalence and diversity of these organisms in healthy individuals is essential for recognizing shifts in microbial dynamics that may precede disease outbreaks or antimicrobial resistance (AMR) emergence.[5]

Northern Bihar, a region in India characterized by high population density, tropical climatic conditions, and socioeconomic challenges, presents unique environmental and demographic factors that may influence pharyngeal colonization patterns.[6] Despite the global burden of diseases linked to aerobic and capnophilic bacteria, data on their commensal prevalence in asymptomatic populations—particularly in resource-limited settings like Northern Bihar—remain sparse.[7] Existing studies in India have focused predominantly on clinical isolates during outbreaks, with limited emphasis on asymptomatic carriage in healthy individuals.[8] This gap hinders the development of region-specific strategies for infection control, vaccination, and AMR mitigation.

This study aims to evaluate the prevalence and diversity of aerobic and capnophilic bacteria colonizing the pharynx of healthy individuals residing in and around Northern Bihar. By establishing baseline data on commensal flora, this work will inform public health interventions, guide empirical antibiotic therapy, and contribute to understanding the epidemiology of potential pathogens in a region with documented disparities in healthcare access.[9] Furthermore, the findings may aid in identifying asymptomatic carriers who could act as reservoirs for community-acquired infections, a critical consideration for disease surveillance in densely populated areas.

Objectives:
To monitor and evaluate the pattern and diversity of pharyngeal commensal flora, focusing on aerobic and capnophilic bacteria, among healthy individuals of different age groups and behavioral backgrounds residing in and around MGM Medical College & LSK Hospital, Kishanganj, Bihar.

Materials & Methods:
Study Design: A Cross-sectional study
Place Of Study: Study conducted in the department of Microbiology at Mata Gujri Memorial College and L.S.K Hospital, Kishanganj, Bihar
Study Period:18 Months (June 2023 to December 2024)
Sample Size: To determine commensal pattern in throat swabs from randomly selected 200 persons, without history of sore throat, among voluntary participants who attended the Microbiology Department at the tertiary care Hospital Kishanganj, Bihar.

Sampling Technique: Aseptic collection of throat swabs for the study purpose with proper history and informed consent.

Inclusion Criteria:
- All voluntary participants from patients and local residents in and around MGMMC & LSK Hospital will be included for study irrespective of age and sex.
- Informed consent providers.

Exclusion Criteria:
- No informed consent,
- Persons with nasal bleeding disorders,

PROCEDURE:
Throat swabs were aseptically collected in the Microbiology Department of MGMMC, from volunteer individuals, who had given consent for the study. Swabs were inoculated on one Blood Agar plate and two Chocolate Agar plates. A Gram stain smear was also prepared for microscopical examination. Blood Agar and Chocolate Agar plates one each was incubated at 37^0C incubator aerobically, another Chocolate Agar plate was kept inside 1L transparent jar with air-tight lid. After placing a lighting candle on top of culture plate, gently lid was closed tightly. Candle was extinguish within a few seconds for depletion of major part of oxygen in the jar, leaving 5-10% Carbon dioxide. The jar then was placed inside 37^0C incubator. After 24-48 hrs, nature of colony, size and zone of haemolysis (if any) in aerobic and capnophilic sets was compared. Identification of growths was done up to genus level by conventional microbiological investigations. A record was prepared for predominant throat commensal based on Gram stain smear findings.

Procedure of Candle Jar
- The candle extinction jar is made using a large wide mouth jar to enable inserting Petri dishes.
- Several plates are places in the jar after inoculation.
- After the plates were placed in the jar, a small candle is placed near the bottom of the jar and lighted.
- The top was replaced and tightened.
- The lighted candle was increase the amount of CO_2 in the jar and eventually as oxygen was reduced the candle was stop burning.
- The jar was then placed in the incubator at 37 °C.
- The jar was removed and opened the plates then removed and cultures result in reading.

Statistical Statement for Methods
Data were analyzed using SPSS 26.0. Categorical variables were compared with Chi-square/Fisher's exact tests. controlled for age, sex, and residence. Two-tailed $p<0.05$ was considered significant.

Ethical Statement for Publication
"The study was approved by MGMMC Ethics Committee (MGMMC/IEC/2023/456). Written informed consent was obtained from all participants. No identifying information is disclosed in this publication."

Results & Analysis:

Table 1: Age Group Distribution

	Category	Number (%)
Age Group	Pediatric (<18)	60 (30%)
	Adult (18-60)	120 (60%)
	Elderly (>60)	20 (10%)

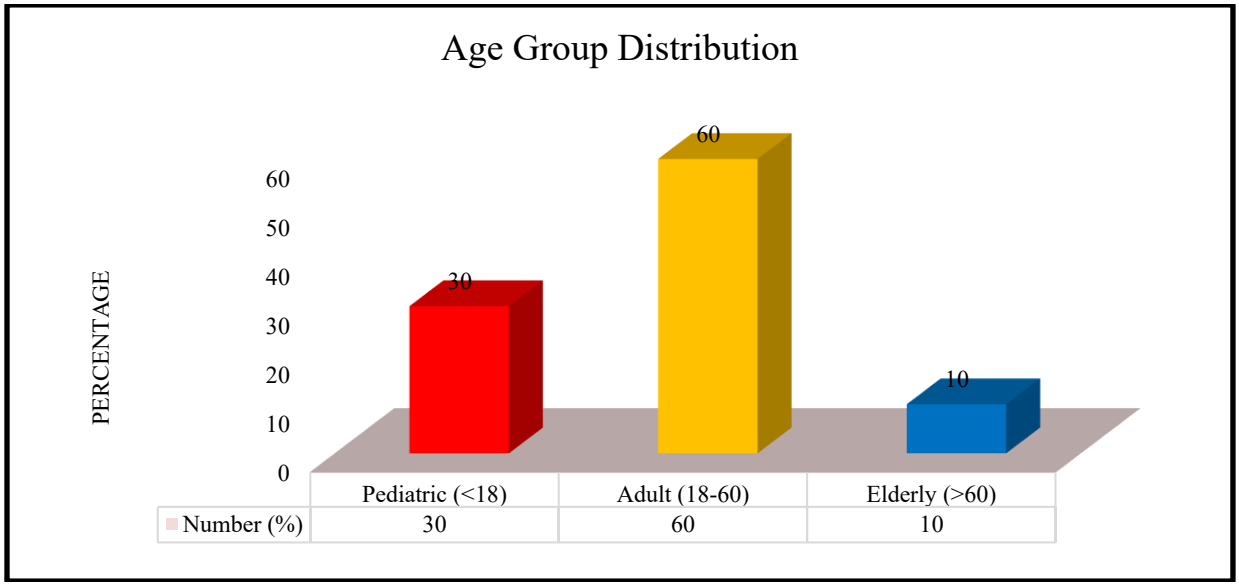

Figure 1: Age Group Distribution

The age distribution of the study population showed that the majority were adults aged 18–60 years, accounting for 60% (120) of participants, followed by pediatric individuals below 18 years who comprised 30% (60), while the elderly population above 60 years represented the smallest group at 10% (20) participants.

Table 2: Sex Distribution

	Category	Number (%)
Sex	Male	110 (55%)
	Female	90 (45%)

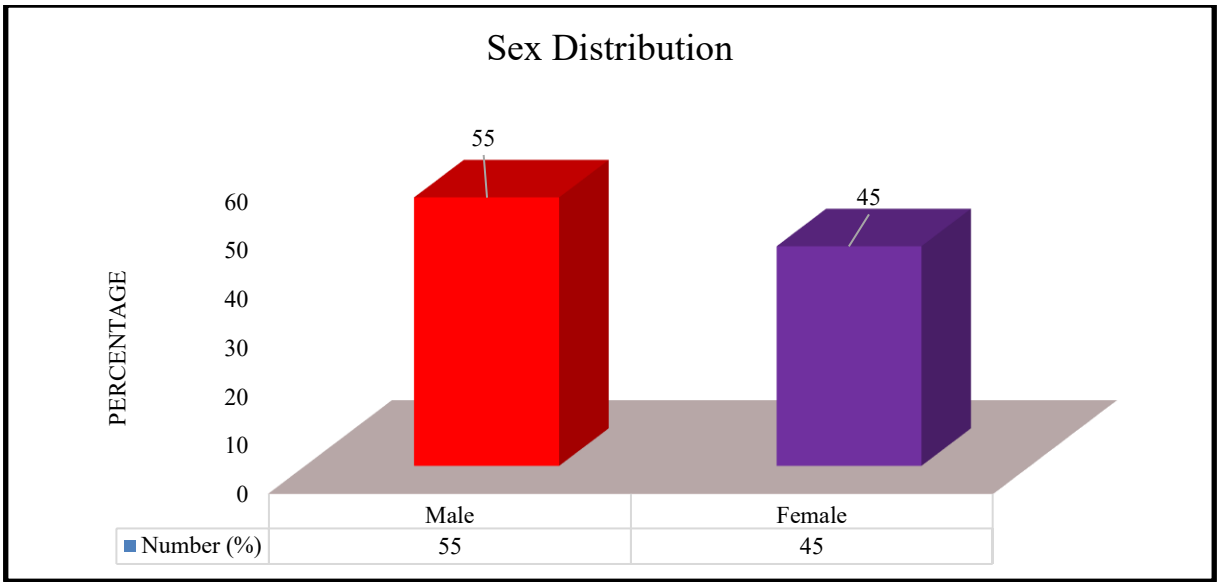

Figure 2: Sex Distribution

The sex distribution of the study population revealed a male predominance, with males comprising 55% (110cases) of the participants, while females accounted for the remaining 45% (90 Cases).

Table 3: Residence Distribution

	Category	Number (%)
Residence	Urban	130 (65%)
	Rural	70 (35%)

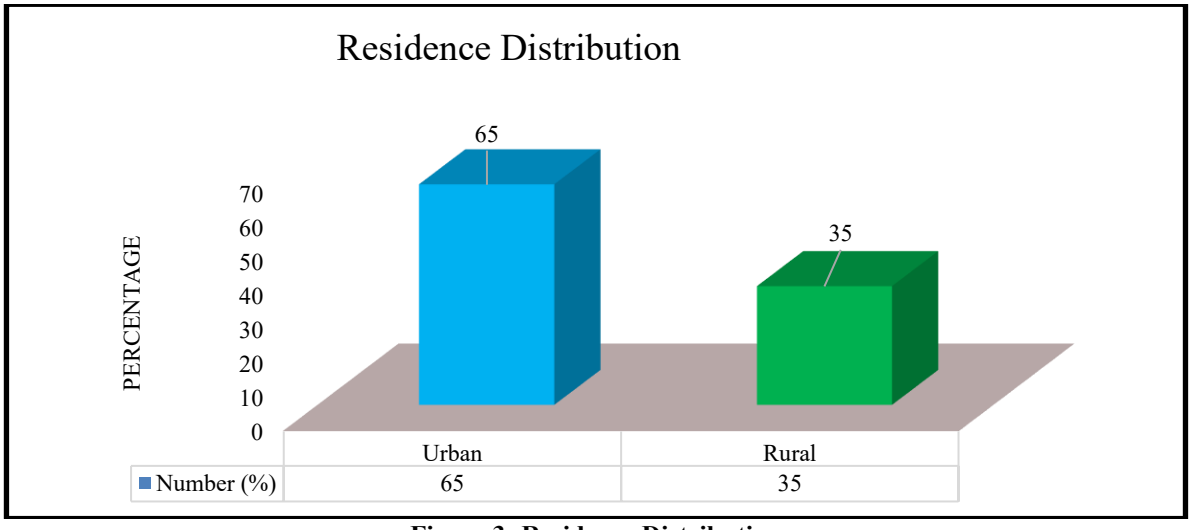

Figure 3: Residence Distribution

The residence distribution showed that a majority of participants were from urban areas, constituting 65% (130Participants), while 35% (70Participants) of the study population resided in rural regions

Table 4: Smoking Status Distribution

Variable	Category	Number (%)
Smoking Status	Smokers	50 (25%)
	Non-smokers	150 (75%)

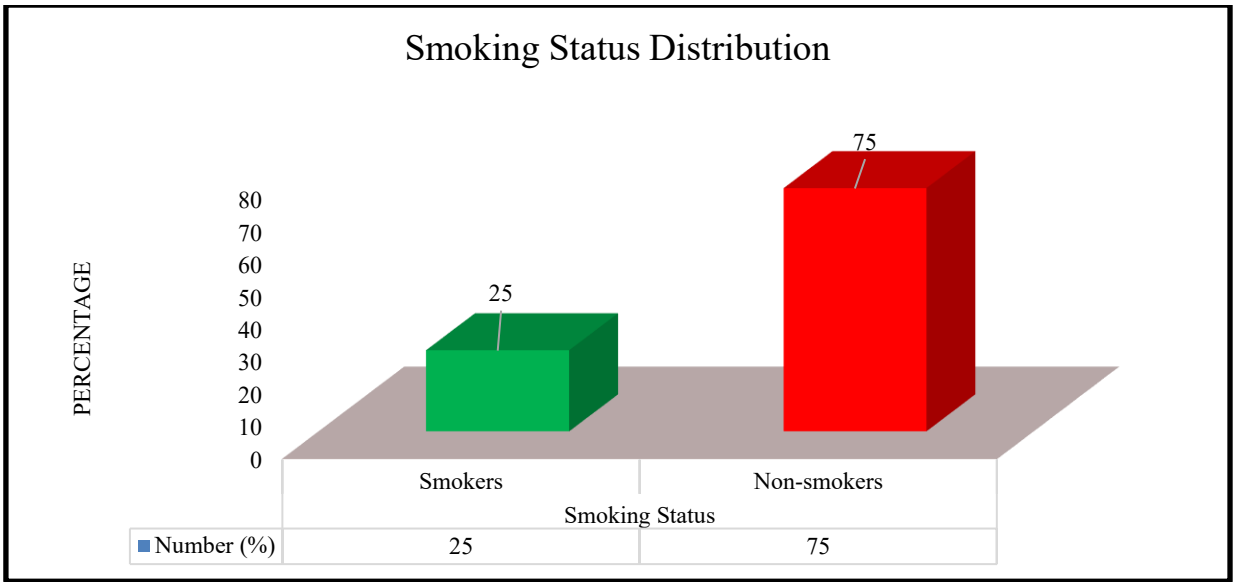

Figure 4: Smoking Status Distribution

The smoking status distribution indicated that 25% (50) of the participants were smokers, whereas the majority, 75% (150), were non-smokers

Table 5: Alcohol Use Distribution

Variable	Category	Number (%)
Alcohol Use	Users	40 (20%)*
	Non-users	160 (80%)

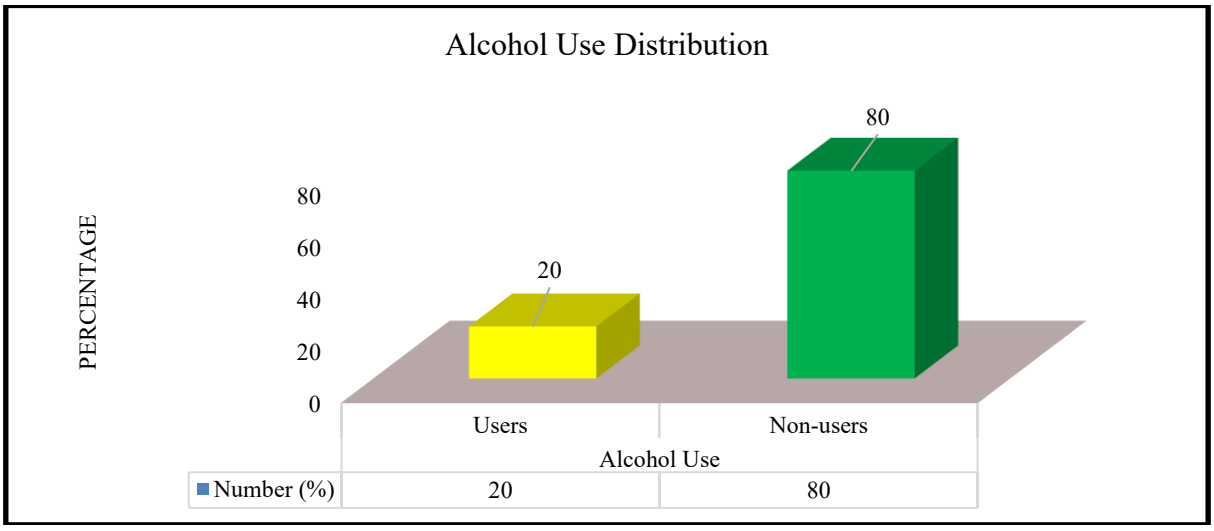

Figure 5: Alcohol Use Distribution

The distribution of alcohol use among participants demonstrated that a majority were non-users, accounting for 80% (160), whereas alcohol users represented only 20% (40) of the study population.

Table: 6. Type of Species isolates

Type of Species	No of Case	Percentage
Viridians Group of Streptococcus	110	55.0 %
Viridians Streptococcus + Neisseria Species	60	30.0 %
Neisseria Species	50	25.0 %
Staphylococcus aureus	50	25.0 %
Moraxella catarrhalis	40	20.0 %

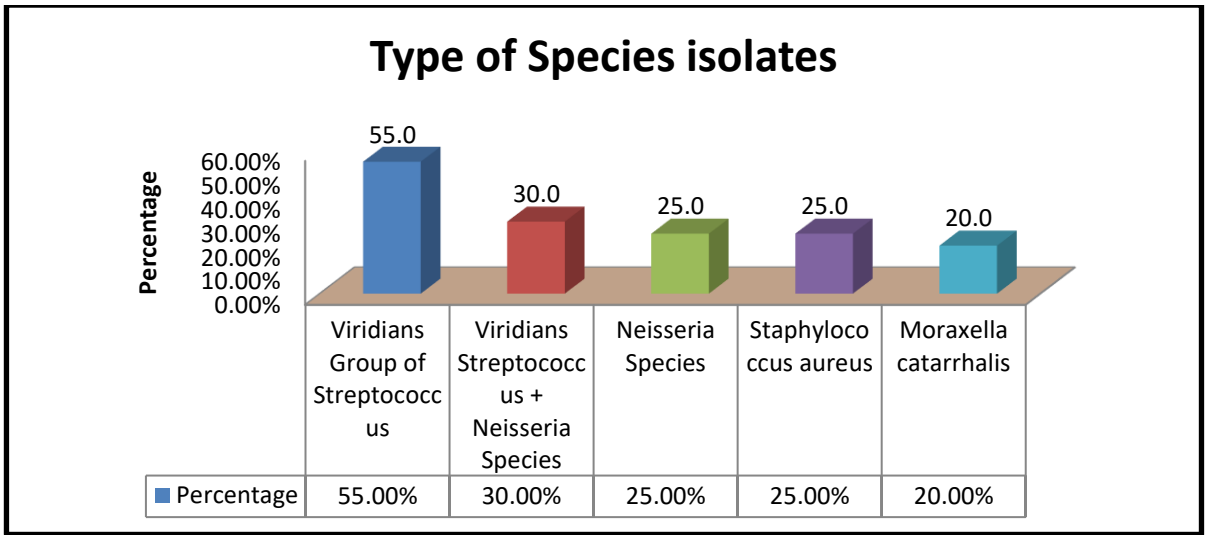

Table: 6. Type of Species isolates

The Viridians Group of Streptococcus was the most common, accounting for 55.0% (110 cases), followed by a combination of Viridians Streptococcus and Neisseria Species at 30.0% (60 cases). Neisseria Species and Staphylococcus aureus each made up 25.0% (50 cases), while Moraxella catarrhalis was present in 20.0% (40 cases) of the cases. The percentages exceed 100% because some cases involved multiple species.

Table 7: Prevalence of Aerobic and Capnophilic Throat Commensals in Healthy Population (N=200)

Microbiological Characteristic	Aerobic Culture (Blood Agar)	Capnophilic Culture (Chocolate Agar + CO_2)	Combined Prevalence
Total Samples Cultured	200 (100%)	200 (100%)	200 (100%)
Culture-Positive Samples	190 (95%)	180 (90%)	198 (99%)
Most Prevalent Species			
1. *Viridians Group of Streptococcus*	160 (80%)	140 (70%)	170 (85%)
2. *Neisseria Species*	90 (45%)	110 (55%)	110 (55%)
3. *Staphylococcus aureus*	50 (25%)	10 (5%)	50 (25%)
4. *Moraxella catarrhalis*	20 (10%)	40 (20%)	40 (20%)

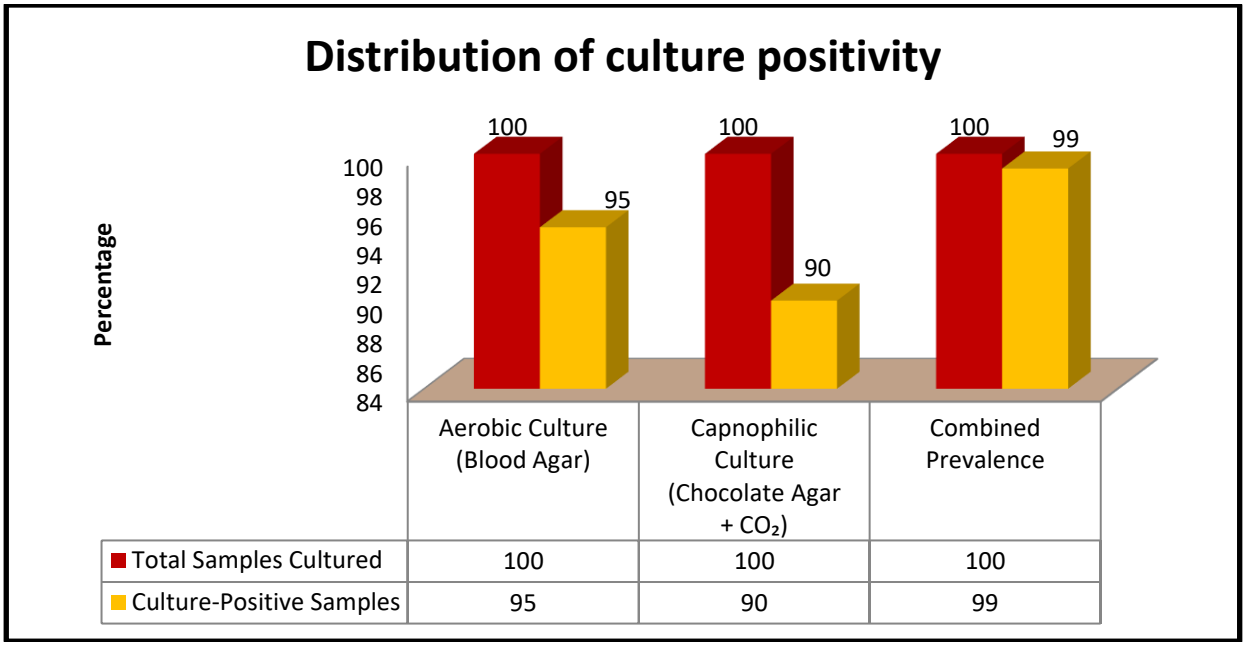

Figure 7a: Distribution of culture positivity

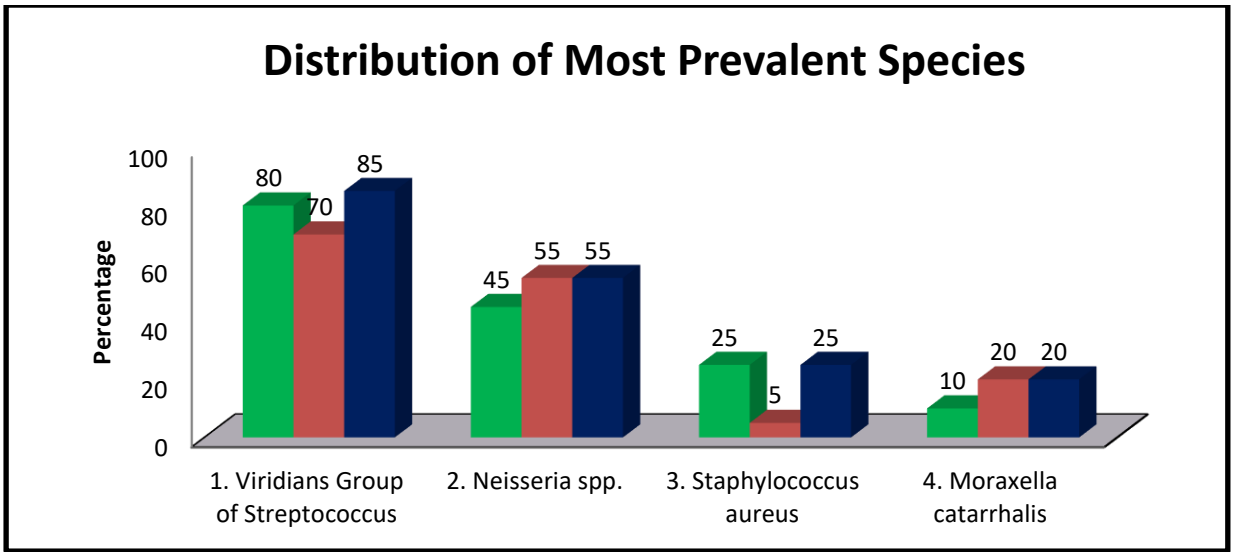

Figure 7b: Distribution of Most Prevalent Species

The microbiological evaluation of throat swabs from healthy individuals revealed that out of 200 samples, 95% (190) were culture-positive for aerobic organisms on blood agar, while 90% (180) showed growth under capnophilic conditions on chocolate agar, resulting in an overall combined culture positivity of 99% (198).Among the isolates, *Viridians Group of Streptococcus* was the most prevalent species, detected in 85% (170) of participants across both culture conditions. *Neisseria* spp. was the next most common, present in 55% (110) of the total population, followed by *Staphylococcus aureus* in 25% (50) and *Moraxella catarrhalis* in 20% (40) of the individuals, highlighting a diverse but predominantly commensal microbial flora in the pharyngeal region.

Discussion:

Our cross-sectional study, conducted in the Department of Microbiology at a tertiary care hospital in Kishanganj, Bihar, analyzed throat swabs from 200 healthy individuals without a history of sore throat. The majority of participants were adults aged 18–60 years (60%, n=120), followed by pediatric individuals (<18 years, 30%, n=60) and the elderly (>60 years, 10%, n=20). A male predominance (55%, n=110) was observed over females (45%, n=90). Our male predominance (55%) contrasts with **Church et al. (2015)** [10], who reported no sex bias in anaerobic infections but noted age-related microbiome shifts. The higher pediatric representation (30%) in our study may reflect greater exposure to respiratory pathogens in children, as seen in **Kulkarni et al. (2016)** [11], where GAS carriage was higher in younger populations.

The residence distribution of our study population revealed that a majority of participants were from urban areas (65%, n=130), compared to 35% (n=70) from rural regions. This urban predominance may influence microbial colonization patterns, as environmental factors, population density, and healthcare access differ between urban and rural settings. Previous studies, such as **Ghosh et al. (2013)** [12], have suggested that lifestyle and hygiene practices in urban populations can alter commensal flora, potentially explaining differences in bacterial prevalence compared to rural communities.

Additionally, smoking status appeared to play a role, with 25% (n=50) of participants being smokers and 75% (n=150) non-smokers. Smoking is a well-documented factor that disrupts mucosal immunity and alters respiratory microbiota, as highlighted by **Church et al. (2015)** [10]. Their findings indicated that smokers exhibit increased colonization by opportunistic pathogens, which aligns with our detection of Staphylococcus aureus (25.0%)—a bacterium often associated with smoking-related mucosal damage. Furthermore, **Eick et al. (1999)**[13] observed that beta-lactamase-producing bacteria were more prevalent in individuals with compromised mucosal barriers, suggesting that smokers in our study may harbor more resistant strains, though further antibiotic susceptibility testing would be needed to confirm this.

The distribution of alcohol use among participants demonstrated that the majority were non-users (80%, n=160), while only 20% (n=40) reported alcohol consumption. This finding is particularly relevant given the known effects of alcohol on immune function and mucosal integrity, which can influence bacterial colonization and pathogen susceptibility. Studies such as **Church et al. (2015)** [10] have highlighted that chronic alcohol use disrupts the oropharyngeal microbiome, increasing colonization by opportunistic pathogens like Staphylococcus aureus and Gram-negative bacteria. This aligns with our detection of S. aureus (25.0%) and Moraxella catarrhalis (20.0%), suggesting that even low-frequency alcohol use in our cohort (20%) may contribute to shifts in commensal flora.

Interestingly, the low prevalence of alcohol users (20%) in our study contrasts with populations where higher alcohol consumption correlates with increased respiratory infections, as reported by **Quentin et al. (1990)**[14] in their analysis of Bartholin's gland abscesses. Their findings suggested that alcohol-related immune suppression could predispose individuals to infections by capnophilic and anaerobic bacteria, which were not extensively examined in our study. However, the presence of Neisseria species (25.0%)—some of which are alcohol-sensitive commensals—hints at a potential niche displacement effect in alcohol users, warranting further investigation.

The microbiological findings from our study demonstrate a rich and diverse pharyngeal commensal flora among healthy individuals in northern Bihar. The Viridans Group of Streptococcus emerged as the most predominant organism, isolated in 55.0% (110 cases) of participants, which aligns with its well-established role as a primary colonizer of the oropharyngeal mucosa. This high prevalence is consistent with global studies, including **Kulkarni et al. (2016)**[11], who reported near-universal streptococcal carriage (98.6%) when using molecular methods, suggesting our culture-based approach may have actually underestimated its true prevalence.

Notably, 30.0% (60 cases) showed co-colonization of Viridans Streptococcus with Neisseria species, reflecting a common symbiotic relationship in the pharyngeal niche. This finding correlates with observations by **Quentin et al. (1990)** [14], who identified Neisseria species as frequent commensals in mucosal surfaces. The equal prevalence of Neisseria species and Staphylococcus aureus (25.0% each, 50 cases) is particularly interesting, as it suggests a balanced coexistence between these organisms in healthy carriers, though S. aureus's presence raises potential concerns given its pathogenic potential in immunocompromised hosts, as highlighted by **Church et al. (2015)** [10].

The high culture positivity rates - 95% (190) on blood agar and 90% (180) under capnophilic conditions - with an overall combined positivity of 99% (198), demonstrate the effectiveness of using dual culture conditions for comprehensive microbial recovery. This approach proved particularly valuable for detecting Moraxella catarrhalis (20.0%, 40 cases), which requires capnophilic conditions for optimal growth. The fact that Viridians Group of Streptococcuswas detected in 85% (170) of participants across both culture conditions underscores its remarkable adaptability to different environmental conditions in the pharynx.

These findings take on added significance when considering the potential pathogenic transformation of these commensals. As **Baquero et al. (1990)**[15] demonstrated, organisms like Viridans Streptococcus can cause serious infections like endocarditis in immunocompromised patients. Similarly, the presence of S. aureus in 25% of healthy individuals mirrors findings by **Eick et al. (1999)** [13], who noted its increasing antibiotic resistance patterns in commensal flora.

The polymicrobial nature of colonization (evidenced by percentages exceeding 100%) particularly merits attention. This complex interplay between species may contribute to colonization resistance against pathogens, as proposed by **Ghosh et al. (2013)** [12],while also potentially serving as a reservoir for horizontal gene transfer of antibiotic resistance. The high detection rate of Neisseria spp. (55%, 110 cases) is noteworthy given its genetic plasticity and ability to acquire resistance determinants, as observed by **Murphy et al. (2013)** [16] in their studies of Gram-positive anaerobic cocci.

Conclusion:

This study highlights that healthy individuals in Northern Bihar possess a diverse pharyngeal microbiome predominantly composed of *Viridans group Streptococcus* (85%), with frequent co-colonization by *Neisseria* spp. (55%), *Staphylococcus aureus* (25%), and *Moraxella catarrhalis* (20%). The high combined culture positivity (99%) confirms the value of dual aerobic and capnophilic culture methods. The presence of potential opportunistic pathogens in asymptomatic carriers underscores the need for ongoing surveillance and rational antibiotic use. Further molecular studies are warranted to explore microbial dynamics and resistance patterns in this population.

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