

# Oropharynx microbiota among alcoholics and non-alcoholics

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**Context:** The oropharynx microbiota plays an important role in the origin of infections, especially among alcoholics whose airway defenses are impaired. **Objective:** To compare the normal oropharyngeal flora in heavy alcohol drinker and non-alcoholics. **Patients:** 117 persons, 58 heavy alcohol drinkers and 59 non-alcoholics. **Setting:** Santa Casa de São Paulo Emergency Service. **Design:** A blind prospective study. **Main outcomes measures:** Prevalence of aerobic and anaerobic bacteria, and fungi. **Results:** The study of the oropharynx microbiota among heavy alcohol drinkers demonstrated the presence of anaerobic microorganisms in 84.5% of them, including: *Bacteroides* sp, *Prevotella melaninogenica*, *Fusobacterium* sp, *Veillonella* sp, *Peptostreptococcus* sp, *Propionibacterium* sp, *Bifidobacterium* sp and *Clostridium* sp, versus 30.5% ( $p < 0.005$ ) of non-alcoholics. *Candida* sp was present in 34.5% of heavy alcohol drinkers and 5.1% of non-alcoholics ( $p < 0.005$ ). Enterobacteria predominated among heavy alcohol drinkers (25%) compared with non-alcoholics (5.5%) only in the age group 14 to 34 years ( $p < 0.05$ ). **Conclusion:** Based upon these results, it was possible to conclude that the knowledge of the oropharynx microbiota among heavy drinkers and non-alcoholics has an important predictive value concerning probable etiologic agents of lower airway infections. Infections caused by anaerobic microorganisms and fungi should be taken into consideration during the choice of empirical therapy for heavy alcohol drinkers.

**UNITERMS:** Alcohol abuse. Oropharynx. Aerobic bacteria. Anaerobic bacteria. Fungi.

## INTRODUCTION

The colonization of the surface of the human oropharyngeal mucous membrane is no easy task for microorganisms<sup>(1)</sup> which must overcome several barriers, among them the fluid flow over the epithelium, mucociliary cleaning, removal of epithelial cells, hosts' inflammatory and immunological responses<sup>(2,3)</sup> as well as the need for an adequate capability to adhere to the mucous cells in order to remain in place.<sup>(4)</sup>

Bacterial adherence to the epithelial cells constitutes an important factor in the colonization of the oropharyngeal mucous membrane because it hinders the elimination of microorganisms, allowing for their growth and development.<sup>(5)</sup>

*Candida* sp. is a commensal microorganism normally found in the human flora; it has been demonstrated in recent years that this microorganism presents a significant capability to adhere to epithelial cells in the vagina, oropharynx, fibronectin, platelet clots, fibrin and vascular endothelium.<sup>(6)</sup> It is possible that, just as with bacteria, adherence plays an important role in the colonization process.

The traditional concept according to which the lungs are permanently sterile has been profoundly modified since Lindsey and Pierce's<sup>(7)</sup> experimental studies. Examining lung tissue from normal dogs, these authors demonstrated

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a low bacterial count, identifying bacteria similar to those found in the resident flora in the animals' pharynx.

On the other hand, it should be stressed that penetration of pharyngeal content into the lower respiratory tract during sleep has been well demonstrated. In fact, bacteria from pharyngeal secretions have been found in the lungs of 45% of normal people, and 70% of patients with a reduced level of consciousness. These interesting studies by Huxley et al<sup>(8)</sup> suggest that inhalation as well as aerosol production may be responsible for the presence of oropharyngeal bacteria within the lungs.

Several mechanisms contribute to avoiding bacterial migration towards the lungs; for instance, the larynx - the natural way to reach the lower lung space - is closed by the glottic reflex during swallowing, hindering inhalation of any material into the lungs. The glottic reflex may be upset in diseases of the central nervous system, during convulsions and the use of central nervous system depressors such as the ethanol abusively consumed by alcoholics, and barbiturates or opiates.<sup>(8,9)</sup>

Coughing constitutes another effective mechanism for expelling material from the lower lung spaces and larynx; for instance, when the cough reflex is disturbed, such as after excessive alcohol ingestion, in neuromuscular diseases, old age, consumptive processes or emaciation, the risk of increased accumulation of secretions drawn from the oropharynx into the lungs may occur.

From what has been said, it may be concluded that the oropharynx microbiota plays an important role in the causation of lungs infections, especially when the defense mechanisms of the hosts' respiratory tract are disturbed.

This is particularly true in the case of heavy alcohol drinkers, in whom the following are common: changes in the normal oropharyngeal flora, decrease of glottic and cough reflexes, diminished function of ciliary cells (hindering the capacity to eliminate secretions from the lower respiratory tract), lower food uptake,<sup>(10)</sup> higher incidence of smoking (predisposing to an increase in secretions which constitute a cultivating medium), and lower inflammatory, humoral and cellular immunity.<sup>(11,12,13)</sup> This whole range of factors undoubtedly contributes to a higher risk of pulmonary infections.<sup>(14)</sup>

In a study of 58 lung infection cases at the Santa Casa Emergency Service via percutaneous transtracheal aspiration, a method developed by Pecora and Brook,<sup>(16)</sup> Golin et al<sup>(15)</sup> identified 85 microorganisms, among which 54.1% were Gram positive aerobic bacteria, 21.2% Gram negative aerobic bacteria, 2.4% Gram positive anaerobics, 7.0% alcohol-acid fast bacilli and 3.5% fungi. The overall incidence of lower airways infection due to anaerobic bacteria was 18.9%, varying from 9% in pneumonia cases up to 66.6% in lung abscesses.

The high rate of infection due to anaerobic bacteria found among lung infection cases and the scarcity of studies concerning the microbial flora in heavy alcohol drinkers motivated the authors to perform the present research aimed at evaluating the oropharyngeal microbiota among heavy alcohol drinkers and comparing it with the microbiota of non-alcoholics.

## METHODS

The present study was undertaken at the Santa Casa de São Paulo School of Medicine Emergency Service involving 117 persons divided into two groups: one of 59 non-alcoholics randomly chosen from among normal accompanying persons, and the other of 58 heavy alcohol drinkers that came for treatment at the Emergency Service.

The ages varied from 14 to 77 years, with an average of 33.07 +/- 13.92 years among non-alcoholics and 39.66 +/- 10.10 among alcohol drinkers. Dividing by gender, non-alcoholics were 54 men and 5 women, and alcohol drinkers were 56 and 2 respectively.

Each one of the participants was informed through personal interview about the procedures to be followed and basic purpose of the study. Consent was elicited before sample collections.

Persons whose medical history disclosed any hospital admission during the previous three months, use of antimicrobial drugs in the preceding four weeks, lung disease, whatever its etiology was, ear or sinus infection or inflammatory process, and lung disease or any other medical condition indicative of recent infectious process, were excluded from both groups.

Heavy alcohol drinkers were defined as patients presenting clinical and biochemical evidence of chronic disease caused by alcohol consumption plus continuous ingestion of over 120 g of ethanol daily during the preceding 12 months, confirmed by medical history.<sup>(17)</sup> According to their medical histories, each of the patients in the alcoholics group was consuming over 500 ml of strong sugar cane alcohol daily.

All samples were collected by the same technician. Sampling was done using alginate sterile cotton swabs vigorously rubbed against the retropharyngeal surface and immediately placed inside a test tube containing 5 ml of thioglycolate broth mixed with 0.5% agar and resazurin as an oxygen indicator.

The sample was inoculated into appropriate media for the cultivation of aerobic and anaerobic bacteria and fungi.

Statistical analysis was performed through chi-square tests to compare percentages and Fisher's test for small size samples. Five percent ( $\alpha=0.05$ ) significance level was accepted in all statistical analyses.

## RESULTS

Among the 58 patients classified as heavy alcohol drinkers, 49 (84.5%) had positive cultures for anaerobic bacteria in samples collected from the retropharynx, while among 59 non-alcoholics, 18 (30.5%) presented anaerobic bacteria. Chi-square test ( $p < 0.05$ ) disclosed a significant difference (Table 1).

There was no statistically significant difference in the cultivation of aerobic bacteria species (Table 1).

Positive results for fungi were found in 20 (34.5%) heavy alcohol drinkers and in only 3 (5.0%) in the non-alcoholic group. Statistical study through the chi-square test ( $p < 0.05$ ) indicated a statistically significant difference between the groups (table 1).

According to the results shown in table 2, Gram positive aerobic bacteria were more frequent among non-alcoholics, the difference being statistically significant. Regarding Gram negative aerobic bacteria, the difference between the groups was not statistically significant. On the other hand, Gram positive and negative anaerobic bacteria predominated among heavy alcohol drinkers, the difference being statistically significant according to the chi-square test ( $p < 0.05$ ) (Table 2).

The study of microorganisms identified among non-alcoholics and heavy alcohol drinkers (Table 3) following statistical analysis demonstrated that neither *Streptococcus viridans* nor *Streptococcus pyogenes* presented a quantitatively significant difference. The same applied to other *Streptococcus* species.

Nor was there a statistically significant difference for *Staphylococcus sp.*, *Enterococcus sp.*, *Neisseria sp.*, *Enterobacteria* and non-glucose fermenting bacteria.

Anaerobic bacteria presented statistically significant differences when prevalences of *Bacteroides sp.*, *Prevotella melaninogenica* and *Bifidobacterium sp.* were compared between non-alcoholics and heavy alcohol drinkers, predominating in the latter group. Other

**Table 1**  
Results of culturing from alcoholics and non-alcoholics

	Non-alcoholics		Alcoholics		Significance testing	
	N	(%)	N	(%)		
<i>Staphylococcus sp</i>	33	55.9	28	48.3	$\chi_1^2 = 0.68$	NS
<i>Streptococcus sp</i>	55	93.2	53	91.4	$\chi_1^2 = 0.14$	NS
<i>Enterobacteria</i>	8	13.5	10	17.3	$\chi_1^2 = 0.30$	NS
<i>Neisseria sp</i>	45	76.3	45	77.6	$\chi_1^2 = 0.03$	NS
Non-Fermenting	2	3.4	2	3.5	$\chi_1^2 = 0.00$	NS
Anaerobics	18	30.5	49	84.5	$\chi_1^2 = 34.81$	S
Fungi	3	5.0	20	34.5	$\chi_1^2 = 16.00$	S
Negative	2	3.4	2	3.5		
Total	59 (RM)	100.0	58(RM)	100.0		

RM- Multiple Responses

$p < 0.05$

**Table 2**  
Bacteria subdivided into non-alcoholic and alcoholic groups according to their tinctorial characteristics

Microorganism	Non-alcoholics		Alcoholics		Significance testing
	N	(%)	N	(%)	
Gram positive aerobic bacteria	100	57.8	87	42.8	$\chi_1^2 = 8.34$ S
Gram negative aerobic bacteria	55	31.8	57	28.1	$\chi_1^2 = 0.62$ NS
Gram positive anaerobic bacteria	4	2.3	17	8.4	$\chi_1^2 = 6.51$ S
Gram negative anaerobic bacteria	14	8.1	42	20.7	$\chi_1^2 = 11.69$ S
Total cultivations	173	100.0	203	100.0	

$p < 0.05$

anaerobic microorganisms did not present statistically significant differences between the groups (Table 3).

It is important to stress that *Candida sp* also predominated among heavy drinkers as evidenced by a statistically significant difference in chi-square testing (Table 3).

Further to the analysis of the role of alcohol, the possible influence of age upon the results was investigated. Both groups were divided into sub-groups, 14 to 34 years, 35 to 54 and over 55. The percentage of anaerobic bacteria and fungi in each age group was calculated.

Significance analysis via the chi-square test (5% critical value, two degrees of liberty) comparing the proportion of occurrence of anaerobic bacteria and fungi, did not reveal any statistically significant difference between any of the groups under consideration.

However, upon examination of the predominance of anaerobic bacteria and/or fungi, comparing non-alcoholics with heavy alcohol drinkers divided between the age categories established, a statistically significant difference for fungi as well as for anaerobic bacteria was identified

in the age group 14 to 34 years, both predominating among heavy alcohol drinkers. In the age group 35 to 54 years, there was a statistically significant difference restricted to anaerobic bacteria. There was no statistically significant difference between non-alcoholics and heavy alcohol drinkers in the age group over 55 (Table 4).

Nor was there any statistically significant difference between the groups concerning staphylococci, streptococci and neisseria.

Enterobacteria were more frequently identified among heavy alcohol drinkers only in the age group 14 to 34, the difference being statistically significant (Table 5).

## DISCUSSION

Many authors<sup>(18-23)</sup> accept knowledge of oropharyngeal microbiota as an excellent indicator for possible agents of bacterial infection of the lower airways,

**Table 3**  
Microorganisms found in the non-alcoholic and alcoholic groups

Microorganism	Non-alcoholics		Alcoholics		Significance testing
	N	%	N	%	
<i>Staphylococcus aureus</i>	16	27.1	10	17.2	$\chi_1^2 = 1.65$ NS
	22	37.3	18	31.0	$\chi_1^2 = 0.51$ NS
<i>Streptococcus</i>	44	74.6	37	63.8	$\chi_1^2 = 1.60$ NS
	02	3.4	02	3.5	$\chi_1^2 = 0.00$ NS
	13	22.0	17	29.3	$\chi_1^2 = 0.81$ NS
<i>Enterococcus sp</i>	03	5.1	02	3.5	$\chi_1^2 = 0.19$ NS
<i>Neisseria Neisseria sp</i>	45	76.3	45	77.6	$\chi_1^2 = 0.03$ NS
<i>Enterobacteria</i>	04	6.8	02	3.5	$\chi_1^2 = 0.67$ NS
	00	0.0	02	3.5	$\chi_1^2 = 2.07$ NS
	01	1.7	01	1.7	$\chi_1^2 = 0.00$ NS
	01	1.7	01	1.7	$\chi_1^2 = 0.00$ NS
	01	1.7	04	6.9	$\chi_1^2 = 1.93$ NS
	01	1.7	00	0.0	$\chi_1^2 = 0.99$ NS
Non-Fermenting	01	1.7	02	3.5	$\chi_1^2 = 0.36$ NS
	01	1.7	00	0.0	$\chi_1^2 = 0.99$ NS
Anaerobics	09	15.3	26	44.8	$\chi_1^2 = 12.20$ S
	02	3.4	09	15.5	$\chi_1^2 = 5.05$ S
	02	3.4	06	10.3	$\chi_1^2 = 2.22$ NS
	01	1.7	00	0.0	$\chi_1^2 = 0.99$ NS
	01	1.7	03	5.1	$\chi_1^2 = 1.07$ NS
	03	5.1	06	10.3	$\chi_1^2 = 1.13$ NS
	00	0.0	05	8.6	$\chi_1^2 = 5.31$ S
	00	0.0	03	5.1	$\chi_1^2 = 3.13$ NS
Fungi	03	5.1	20	34.5	$\chi_1^2 = 16.00$ S
Total	59 (MR)	100.0	58 (MR)	100.0	

MR- Multiple Responses

**Table 4**  
**Presence of anaerobics and fungi in different age groups among non-alcoholics and alcoholics**

Age groups	Non-alcoholics					Alcoholics					Significance testing	
	Total	Anaerobics		Fungi		Total	Anaerobics		Fungi		f/Anaerobics	f/Fungi
		N	(%)	N	(%)		N	(%)	N	(%)		
14-34	36	10	27.8	2	5.5	16	15	93.0	4	25.0	$\chi_1^2 = 5.99$ S	$\chi_1^2 = 4.10$ S
35-54	16	5	31.3	3	18.8	37	29	78.3	16	43.3	$\chi_1^2 = 10.79$ S	$\chi_1^2 = 2.91$ NS
55 or more	7	3	42.8	0	—	5	5	100	—	—	$\chi_1^2 = 2.09$ NS(cc)	$\chi_1^2 = 0.00$ NS
Total	59	18	30.5	20	34.5	58	49	84.5	20	34.5		

cc= corrected

**Table 5**  
**Presence of enterobacteria in different age groups among non-alcoholics and alcoholics**

Age groups	Non-alcoholics			Alcoholics			Significance testing
	Total	enterobacteria		Total	enterobacteria		
		N	(%)		N	(%)	
14-34	36	2	5.5	16	4	25.0	$\chi^2 = 4.10$ S
35-54	16	4	25.0	37	4	10.8	$\chi^2 = 2.22$ NS
55 or more	7	2	28.6	5	2	40.0	$\chi^2 = 0.04$ (cc)NS
Total	59	6	10.2	58	10	17.3	

p<0.05

therefore allowing physicians to adequately prescribe antimicrobial therapy avoiding indiscriminate use of antibiotics, singly or in association, which as well as being harmful are expensive for the patients and the community.

Considering the high prevalence of alcoholism, infectious complications in heavy drinkers assume the utmost importance. In fact, Nolan,<sup>(24)</sup> in a prospective evaluation of 900 hospital admissions observed an incidence of bacterial pneumonia equal to 16% among alcoholics and 6.5% in non-alcoholics.

According to Schmidt & De Lint,<sup>(25)</sup> mortality among alcoholics with pneumonia was three times higher in men and seven times in women, when compared with non-alcoholics.

Several mechanisms justify the higher incidence of pneumonia among alcoholics and, in addition, they are prone to inhale oropharyngeal material contaminated by microorganisms while asleep and during episodes of loss of consciousness.<sup>(8,9,26,27)</sup>

This possibility is supported by studies proving the importance of inhalation of oropharyngeal secretion by alcoholics, as well as by those showing that the amount of inhaled bacteria is also a fundamental factor in the etiopathogenesis of bacterial pneumonia.<sup>(28,29,30,31)</sup>

The special significance of the present paper is that, contrary to what is indicated in the references surveyed, the simultaneous presence in the oropharynx of aerobic and anaerobic bacteria and fungi is analyzed. This has

relevance because in the discussion of the proportional constitution of a system, if all of the constituting elements are not considered, evidently, the proportions will not be representative of the system under consideration. It is not possible, for instance, to confirm enterobacteria as predominant components of the heavy alcohol drinkers' normal microorganism flora, if we do not include all of the component microorganisms of the flora, ie aerobics, anaerobics and fungi, in the examination. Moreover, this knowledge will help physicians to decide upon treatment when there is a possibility of infection of the lower airways due to the inhalation of pharyngeal contents.

When we demonstrated<sup>(15)</sup> via transtracheal puncture that 18.9% of patients had lung infections caused by anaerobic bacteria, attention was drawn to the fact that references consulted up until that time did not show a single study on the subject in the country. The question was then raised as to whether many patients were being inadequately treated because the simultaneous presence of anaerobics and/or fungi was not being considered as a possibility. Another fact not studied up until that time was the observation that heavy alcohol drinkers were among those most susceptible to infection by anaerobic microorganisms in respiratory infectious diseases.

Taking into consideration several studies stressing knowledge of the normal oropharyngeal flora as an important predictive factor for pulmonary infection cases,<sup>(18-23)</sup> the authors proceeded to make a search among heavy alcohol drinkers, since they could harbour a higher

number of anaerobic bacteria in the oropharynx, which could be an important etiopathogenic factor in such infections.

In the present study, 84.5% of alcoholics were identified as having anaerobic bacteria in the oropharynx, against 30.5% in non-alcoholics. These finds are relevant because they are indicative, for the physician, of possible etiologies for lower airway infections in patients arriving at an Emergency Service (Table 1).

Gram positive and Gram negative anaerobic bacteria were found with a higher frequency among alcoholics (Table 2); this finding is important, especially when the lack of sensitivity of *Bacteroides sp* to the antibiotics often employed in the treatment of pneumonia is considered.

In addition to alcohol abuse, the meaning of the age factor was also evaluated as a determinant in the incidence of specific bacteria in the colonization of the oropharynx. It was possible to conclude, according to statistical analysis, that there was no significant difference when age groups were compared, and between alcoholics and non-alcoholics as well. In conclusion, age factor had no influence in the prevalence of anaerobics in either of the two categories under study (Table 4).

However, comparing age ranges in the two groups, non-alcoholics and heavy alcohol drinkers, a higher frequency of anaerobics among alcoholics aged 14 to 34 and 35 to 54 (Table 4) was detected. No statistically significant difference was revealed between the groups in the age category of over 55.

Another important finding was the higher proportion of *Candida sp* in the oropharynx of heavy drinkers compared with non-alcoholics: 34.5% against 5.0% (Table 3). Knowledge of this result, in spite of the scarcity of such pathogens as agents of lung infection in persons free of acquired immunodeficiency syndrome, becomes relevant in heavy drinkers. These microorganisms are seldom

considered in the clinical routine of Emergency Services, in spite of the fact that they produce severe infections. Since they are not affected by the usual antimicrobial drugs, their action inside the lungs will continue, leading to a gradual deterioration of the patient's state of health, perpetuating the infection, aggravating the clinical condition and finally leading to death. Davies<sup>(32)</sup> refers to inhalation of oropharyngeal secretions as an important factor in the pathogenesis of pneumonia caused by *Candida sp*.

Analysis of the behaviour of fungi among heavy drinkers and non-alcoholics shows a difference restricted to the age group 14 to 34 years, with predominance among alcoholics. Alcoholics in the age group 14 to 34 years present higher colonization by *Candida sp* and are therefore more susceptible to infection by this microorganism.

Microorganisms that make up the oropharyngeal flora of alcoholics become important pathogenic agents of infection of the lower airways when mechanical, immunological and inflammatory defenses in these patients are severely affected.<sup>(33)</sup> When alcoholics inhale large amounts of oropharyngeal secretions containing significant amounts of bacteria able to produce infectious diseases, we need to acknowledge and emphasize that the activity of anaerobic germs and fungi should be considered, being especially alert to the possibility of multi-microbial infections, and therefore we should direct specific treatment accordingly.

In conclusion, it may be affirmed that oropharynx microbiota in heavy drinkers show a predominance of anaerobic bacteria and *Candida sp* when compared with non-alcoholics

This fact indicates that physicians must keep in mind the possible presence of such agents producing lower airway infections before deciding on which drugs to employ, so as to prescribe appropriate treatment, especially when patients are not progressing satisfactorily.

## RESUMO

**Contexto:** O microbiota da orofaringe assume importante papel na gênese das infecções, principalmente dos alcoólatras, cujas defesas do trato respiratório estão comprometidas. **Objetivo:** Comparar a flora normal da orofaringe em alcoólatras pesados e não-alcoólatras. **Tipo de estudo:** Estudo cego e prospectivo. **Participantes:** 117 indivíduos - 58 alcoólatras pesados e 59 não-alcoólatras. **Local:** Serviço de Emergência da Santa Casa de São Paulo. **Variável estudada:** Prevalência de bactérias aeróbias, anaeróbias e fungos. **Resultados:** O estudo do microbiota da orofaringe de alcoólatras pesados comparado com o dos não alcoólatras, evidenciou 84,5% de microrganismos anaeróbios - *Bacteroides sp*, *Prevotella melaninogenica*, *Fusobacterium sp*, *Veilonella sp*, *Peptostreptococcus sp*, *Propionibacterium sp*, *Bifidobacterium sp* e *Clostridium sp* contra 30,5% ( $p < 0,05$ ). A *Candida sp* esteve presente em 34,5% dos alcoólatras pesados e em 5,1% dos não alcoólatras ( $p < 0,05$ ). As enterobactérias predominaram nos alcoólatras pesados (25%) em relação aos não alcoólatras (5,5%), apenas na faixa etária entre os 14 e 34 anos ( $p < 0,05$ ). **Conclusão:** Estes resultados permitiram concluir que o conhecimento do microbiota da orofaringe de alcoólatras pesados e não alcoólatras, tem importante valor preditivo, quanto aos prováveis agentes etiológicos das infecções das vias aéreas inferiores. As infecções por microrganismos anaeróbios e fungos devem ser consideradas durante a escolha da terapêutica empírica nos alcoólatras pesados.

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