v. 4, n. 104, 2024

International Journal of Health Science

EVALUATION OF THE AEROBIC MICROBIOTA OF THE OROPHARYNX OF PATIENTS HOSPITALIZED FOR ELECTIVE HEAD AND NECK SURGERY

Acceptance date: 13/12/2024

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Abstract: Introduction: The microbiota refers to the population of microorganisms that inhabit the skin and mucous membranes of healthy people and can be classified as resident or transient. In terms of quantity and quality, it can be said that the microbiota is not uniform, as each region has a microbiota with its own characteristics. **Aim: To** identify the aerobic oropharyngeal microbiota of patients hospitalized for elective head and neck surgery at the time of hospital admission and to see if there were any changes in this microbiota 48 hours after admission. **Method:** 99 samples of secretion from the posterior wall of the oropharynx were collected from hospitalized patients using a sterile bacteriological *swab* or loop. The samples were collected on admission and 48 hours after hospitalization and submitted to microbiological analysis. **Results**: 64.64% of the patients showed a change in the aerobic microbiota of the oropharynx after 48 hours of hospitalization. **Conclusion:** *Candida albicans, Candida tropicalis, Enterobacter aerogenes, Escherichia coli, Klebsiela oxytoca, Klebsiella pneumoniae, Micrococcus* spp, *Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Staphylococcus coagulase negative and Streptococcus viridans* were identified in the oropharyngeal microbiota of the research participants and 64.64% of the individuals studied showed changes in their microbiota.
Keywords: microbiology, oropharynx, bacterial adherence, microbiota.

INTRODUCTION

The population of micro-organisms that is frequently found on a certain surface of the human body and which easily recomposes itself is called the resident microbiota. It is acquired after birth, as contact with microorganisms occurs, and has the function of preventing colonization by pathogens[1,2]. Most of this microbiota is made up of bacteria, but fungi and other microorganisms are also found[3]. Some protozoa can also be part of the human resident microbiota, but in smaller quantities than bacteria.[4]

In terms of quantity and quality, it can be said that the microbiota is not uniform, as each region has a microbiota with its own characteristics[5].

The microorganisms that make up the resident microbiota do not cause disease under normal health conditions.[6]

The oral cavity is the main gateway for microorganisms from the external environment to enter the human body, for example through chewed food and even inhaled air. Microorganisms that colonize a certain area of the oral cavity have a good chance of spreading to contiguous epithelial surfaces of neighbouring sites.[7]

The transient microbiota consists of microorganisms that remain on the skin or mucous membranes for hours, days or weeks, coming from the external environment and not establishing themselves permanently there. In general, members of the transient microbiota are of little significance as long as the resident microbiota remains intact; however, if the resident microbiota is altered, the transient microorganisms can colonize and multiply, potentially causing disease.[1,2]

In healthy adults, the microorganism that predominates in the oral cavity is Streptococcus viridans, but the oral resident microbiota in critically ill patients changes and becomes predominantly Gram-negative microorganis-

ms, constituting a more aggressive microbial population, which can be composed of *Acinetobacter baumannii, Haemophilus influenzae, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae and Enterobacter spp.*[8]

Among the microorganisms most frequently isolated from the oropharynx are *Staphylococcus aureus*, *Streptococcus pneumoniae*, as well as Enterobacteria and some non-glucose fermenting Gram negative bacilli[9]. It is worth mentioning that *Streptococcus pneumoniae* can have its growth inhibited on agar chocolate (a widely used culture medium) due to the accumulation of hydrogen peroxide[10].

The microorganisms of the oropharyngeal microbiota are important sources of infections, especially among people whose airway defenses are impaired by anatomical changes, age and immune weakness, use of alcohol, drugs and tobacco.[7]

Some bacteria capable of causing pharyngitis require special technical conditions to isolate them, such as *Corynebacterium diphtheriae, B. pertussis, N. gonorrhoeae*, species of *Chlamydia* spp and *Mycoplasma pneumoniae.*[2]

There is a predominant increase in *Streptococus mutans, Actinomyces naeslundi* and lactobacilli in the oral microbiota after radiotherapy in this region, which is explained by the changes suffered by the salivary glands during this type of treatment.[11]

Other very pathogenic bacteria such as *Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae*, Enterobacteriaceae and *Pseudomonas* species rarely cause oropharyngeal infection, although they are found in this region of some people[12]. These bacteria are considered very pathogenic, but rarely cause infection in this region[2].

Patients irradiated in the head and neck region show a clear change in the oral resident microbiota, with an increase in the population of *Staphylococcus aureus* and coliforms, as well as *Candida albicans.*[13]

Some physical and chemical factors influence the growth of microorganisms and consequently the composition of the resident microbiota, such as pH, the presence of oxygen and exposure to sunlight. Some other factors that affect the resident microbiota are: age, nutritional status, hospitalization, diet, state of health, existence of disabilities, emotional state, stress, climate, geographical location, personal hygiene conditions, occupation and lifestyle and socioeconomic conditions[6]. Alcoholism, smoking and reduced salivary flow are also factors that can influence the increase or decrease in the oral resident microbiota[8]. Hospitalization is also one of the factors that can alter the resident microbiota.[14]

The use of antimicrobials has been an important factor associated with the modification of the oral microbiota, and can even induce rapid changes[15,16]. The use of these drugs can cause a reduction in the number of microorganisms inhabiting the resident microbiota and allow excessive growth of other species already present.[15]

With regard to length of stay, Amaral[8] reports that 48 hours after admission to the intensive care unit, all the patients in the study had colonization of the oropharynx by Gram-negative bacilli.

Reducing hospitalization time for elective surgery, for example, can help patients avoid acquiring pathogenic microorganisms from the hospital environment.

The importance of this work lies in showing possible changes in the resident microbiota, from the possible acquisition of pathogenic microorganisms, probably inserted as a result of prolonged hospitalization.

OBJECTIVE

General objective: To identify the aerobic oropharyngeal microbiota of patients hospitalized for elective head and neck surgery on admission and 48 hours later. Specific objective: To check whether there was a change in the composition of the aerobic microbiota of the oropharynx 48 hours after hospitalization compared to the aerobic oropharyngeal microbiota identified on admission.

METHOD

Over a period of one year, 99 samples of oropharyngeal secretions were collected from patients hospitalized at the Hospital do Servidor Público Estadual de São Paulo (IAMSPE) for elective head and neck surgery, these being consecutive patients with no previous treatment. This study was approved by the institution's research ethics committee (CAAE 32520414.3.0000.5463) with opinion number 718.395 on 08/07/2014. A Free and Informed Consent Form (FICF) was drawn up and the participants were duly instructed about the research.

There was no selection related to age, gender, color, ethnicity, pre-existing diseases, use of medication or type of surgery for the collection of oropharyngeal secretions. All the participants had elective surgery previously scheduled by the head and neck surgery service.

This is an experimental project with no financial resources from corporate grants.

Samples were taken on the day of hospitalization and 48 hours after admission, before surgery and before receiving prophylactic antibiotic therapy. The biological material was obtained using a cotton-tipped swab with a flexible plastic rod from the Absorve® brand or a plastic bacteriological loop with a flexible rod from the Lab Plast® brand.

Sterilized by ethylene oxide. The patient was asked to open their mouth while the swab or bacteriological loop was introduced into the patient's oral cavity, always taking great care not to touch any surface other than the oropharynx. With the help of a sterile wooden tongue depressor, the tongue was prevented from being touched by the swab or the loop and the secretion from the posterior wall of the oropharynx was then captured using gentle rotating movements.

It was decided as a protocol not to collect samples from patients who had used toothpaste or mouthwash for at least 2 hours prior to collection, as these products could contain germicidal substances in their composition, and it was also established that patients who had excessive food residue adhered in the oral cavity should simply rinse with water only, The swab or bacteriological loop was carefully inserted without touching any surfaces in the oral cavity other than the oropharynx.

The material was sown by streaking on the surface of Himédia® chocolate agar. The samples were then incubated in an aerobic oven at 35-37 degrees Celsius (°C), remaining under these conditions for a period of 48 hours.

As for the identification of the microorganisms, we followed the guidelines set out in the manual for the detection and identification of bacteria of medical importance issued by the National Health Surveillance Agency (ANVISA)[17]. The macro and micro morphological criteria of the colonies were followed, as well as the biochemical identification tests.

Colonies suggestive of *Candida* spp were identified through their macro and micro morphological characteristics and development on selective Saboureau Dextrose agar with chloramphenicol from Himédia® and on Chromagar from Laborclin®, a specific agar for identifying Candida species.

The bacterial colonies grown on selective media were also analyzed for their isolation purity using Gram staining, and these were only inoculated onto biochemical identification media if they were free from contamination by another microorganism of a different morphology and color characteristic.

The microorganisms that developed in the admission culture and in the culture 48 hours after hospitalization were noted and then compared to see if there had been a change in the composition of the microbial members.

Changes were computed as insertion or subtraction changes, where insertion changes included a certain microorganism that was not present in the admission culture, but was present in the culture after 48 hours of hospitalization, and subtraction changes included the absence of the microorganism in the 48-hour culture, but which was present in the admission culture.

The statistical analysis was descriptive and no software was used. The graphs and tables come from Microsoft office® Word 2010.

RESULTS

Of the 99 study participants, 69 were women. The patients ranged in age from 20 to 88, with the average age being 58.

All patients admitted for elective head and neck surgery received antibiotic prophylaxis only at the time of surgery. There were no reports of antibiotics being administered between the time of admission and 48 hours after hospitalization. There is no information on the use of antibiotics by patients in the months prior to hospitalization.

None of the participating patients reported brushing their teeth with toothpaste and/or gargling with mouthwash for at least 2 hours prior to collection.

No excess food residue adhered to the oral cavity of any of the patients participating in the study.

Type of surgery	"n"	Percentage
Oral cavity	5	5,05%
Oral cavity + cervical emptying	1	1,01%
Correction of tracheocutaneous fistula	1	1,01%
Cervical drainage	8	8,08%
Salivary gland extraction	7	7,07%
Laryngectomy + Neck dissection	1	1,01%
Parathyroidectomy/Thyroidectomy	59	59,59%
Parathyroidectomy/Thyroidectomy + Neck dissection	3	3,03%
Laryngectomy	5	5,05%
Cervical cyst resection	2	2,02%
Cervical cyst resection + salivary gland extraction	1	1,01%
Resection of cervical metastasis	1	1,01%
Resection of skin neoplasms	4	4,04%
Nasal floor resection + oral cavity surgery	1	1,01%

Table 1: Distribution of patients in relation to the surgical procedures they underwent

64.64% of patients had changes in their microbiota after 48 hours.

The 64 individuals who showed a change in aerobic microbiota of the oropharynx had a minimum age of 22 and a maximum age of 83, with a mean age of 61.

Among the 30 males, 21 of them, i.e, 70% showed a change in oropharyngeal microbiota 48 hours after hospitalization. Of the 69 women who took part in the study, 43 (62.3%) showed a change.

Type of surgery	"n"	Percentage
Oral cavity	6	9,37%
Correction of tracheocutaneous fistula	1	1,56%
Cervical drainage	11	17,18%
Salivary gland extraction	7	10,93%
Laryngectomy	3	4,68%
Parathyroidectomy/Thyroidectomy	34	53,10%
Cervical cyst resection	1	1,56%
Resection of cervical metastasis	1	1,56%
Resection of skin neoplasms	4	6,25%
Resection of the nasal floor	1	1,56%

Table 3: Description and distribution of the type of surgery scheduled among the 64 patients who had a change in the aerobic microbiota of the oropharynx 48 hours after hospitalization

Note: Some patients underwent surgery on more than one anatomical site in the same operation.

Change by insertion and subtraction of a given microorganism	Modification only by inserting a particular microorganism	Change only by subtraction of a certain microorganism
18 patients (28,12%)	22 patients (34,37%)	24 patients (37,50%)

Table 4: Categorization of changes in the aerobic microbiota of the oropharynx after 48 hours of hospitalization and number of patients in each category (of the 64 individuals who showed changes)

DISCUSSION

Murray[2] and Brooks[3], report that most of the resident microbiota is made up of bacteria, but fungi and other microorganisms are also found. In this study, of all the types of microorganisms isolated, the majority were bacteria, meaning that the results obtained show the same. The authors cited above also report that the human transient microbiota consists of micro-organisms that remain on the skin or mucous membranes for hours, days or weeks, come from the external environment, do not settle permanently in the area, have a short survival time and are easily transmitted by contact. In this study, the microorganisms that were found in the admission culture and then were no longer found can be attributed to the transient microbiota. The same may have occurred with those that were only found in the culture after 48 hours of hospitalization.

In healthy adults, the microorganism that predominates in the oral cavity is *Streptococcus viridans*, but the oral microbiota resident in the patients in critical health changes and becomes predominantly Gram-negative microorganisms, constituting a more aggressive microbiota, among them are *Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae and Enterobacter spp*[8].

Most of the patients studied had *viridans group Streptococcus* in the oropharyngeal secretion cultures, both on admission and after 48 hours of hospitalization, data which is similar to Amaral[8] who reports *viridans* group *Streptococcus* as the predominant microorganism in the oral cavity. The results show that there are cases of *Pseudomonas aeruginosa and Klebsiella pneumoniae* inserted into the cultures after 48 hours of hospitalization, but *Proteus mirabilis* and *Enterobacter aerogenes* were found in the admission culture and then subtracted after 48 hours, which may mean transient microbiota in the admission culture. In relation to the findings of these Gram-negative bacteria mentioned in this paragraph, Murray[12] reports that these pathogenic microorganisms can be found in the oropharynx of some people without causing infection in this region.

Hospitalization is one of the factors that can change the resident microbiota, say the authors[6,14]. In this study, there was a change in the oropharyngeal microbiota of 64.64% of the hospitalized patients studied.

According to Oplustil[9], among the microorganisms most frequently isolated from the oropharynx are *Staphylococcus aureus*, *Streptococcus pneumoniae*, as well as Enterobacteria and some non-glucose fermenting Gram negative bacilli. Among the non-glucose fermenting Gram negative bacilli isolated in this study, only *Pseudomonas aeruginosa* was isolated, although there were growth conditions for other genera and species of this type of bacillus. According to Murray[10], *Streptococcus pneumoniae* can have its growth inhibited due to the accumulation of hydrogen peroxide, as observed in chocolate agar. This may be an explanation for the absence of this bacterium in the results of this study, as the agar used for the cultures was the same.

Murray[2] reports that some very pathogenic bacteria, such as *Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae*, Enterobacteriaceae and *Pseudomonas* spp are found in the oropharynx of some people, but rarely cause infection in this region. Of the bacteria cited in the literature described

Micro-organism identified	"n" of admission culture	Percentage the culture of admission	"n" of the 48-hour culture after admission	Percentage of culture 48 hours after admission
Candida albicans	19	19,19%	15	15,15%
Candida tropicalis	1	1,01%	0	0%
Enterobacter aerogenes	1	1,01%	0	0%
Escherichia coli	3	3,03%	2	2,02%
Klebsiela oxytoca	0	0%	3	3,03%
Klebsiella pneumoniae	5	5,05%	4	4,04%
***Micrococcus* spp**	10	10,10%	11	11,11%
Proteus mirabilis	2	2,02%	1	1%
Pseudomonas aeruginosa	2	2,02%	4	4,04%
Serratia marcescens	2	2,02%	3	3,03%
Staphylococcus aureus	25	25,25%	33	33,33%
Coagulase negative Staphylococcus	49	49,49%	33	33,33%
Streptococcus viridans group	98	98,98%	96	96,96%

Table 2: Distribution of patients detected with a given microorganism in the culture of oropharyngeal secretion on admission and 48 hours after hospitalization

Note: Several patients had more than one type of microorganism.

Microorganism	"n" of modification by insertion	Percentage change per insertion	"n" of modification by subtraction	Percentage change by subtraction
Candida albicans	**4**	**6,25%**	**8**	**12,50%**
Candida tropicalis	0	0%	1	1,56%
Enterobacter aerogenes	0	0%	1	1,56%
Escherichia coli	**0**	**0%**	**1**	**1,56%**
Klebsiela oxytoca	**3**	**4,68%**	**0**	**0%**
Klebsiella pneumoniae	4	6,25%	3	4,68%
***Micrococcus* spp**	**4**	**6,25%**	**3**	**4,68%**
Proteus mirabilis	**0**	**0%**	**1**	**1,56%**
Pseudomonas aeruginosa	3	4,68%	1	1,56%
Serratia marcescens	2	3,12%	1	1,56%
Staphylococcus aureus	16	25,00%	9	14,06%
Staphylococcus coagulase negative	10	15,62%	22	34,37%
***Streptococcus viridans* group**	2	3,12%	1	1,56%
Total cases	48	74,97%	52	81,21%

Table 5: Distribution of changes by insertion and subtraction of the aerobic microbiota of the oropharynx for each microorganism in patients hospitalized for elective head and neck surgery

Note: More than one type of microorganism was found in several of the individuals studied.

35 individuals showed no change in the oropharyngeal aerobic microbiota after 48 hours of hospitalization. These patients ranged in age from 20 to 88, with a mean age of 55.

above, *Staphylococcus aureus* and *Pseudomonas* spp (the *aeruginous* species) were found and only *Streptococcus pneumoniae and Haemophilus influenzae* were not isolated.

Some bacteria capable of causing pharyngitis require special technical conditions to isolate them, such as *Corynebacterium diphtheriae, B. pertussis, N. gonorrhoeae, species* of *Chlamydia spp.* and Mycoplasma *pneumoniae. gonorrhoeae*, species of Chlamydia spp and *Mycoplasma pneumoniae*, says Murray[2], but the culture media and/or incubation time used in this study did not favor the development of *Bordetella pertussis and Corynebacterium diphtheriae*, nor did it allow the diagnosis of *Chlamydia spp* and *Mycoplasma pneumoniae.*

Amaral[8] mentions that 48 hours after admission to an intensive care unit, all the patients in a study had colonization of the oropharynx by Gram-negative bacilli. In this study, only a small proportion of the patients who had a change in the aerobic microbiota of the oropharynx had Gram-negative bacilli inserted. It is worth pointing out that the patients were staying in a common ward, and not in an intensive care unit.

According to Savage[14], the use of antibiotics can influence the composition of the oral microbiota and Palone[15] reports that there can be changes in the resident microbiota of the oropharynx after antimicrobial therapy, there can be a reduction in the number of microorganisms that inhabit the resident microbiota and allow excessive growth of other species already present. According to Cardoso[1] and Murray[2], in general, members of the transient microbiota are of little significance as long as the resident microbiota remains intact; however, if the resident microbiota undergoes changes, transient microorganisms can colonize. In relation to what the authors mention above, the use of antibiotics in the period prior to the second collection of this work scientific research could alter the composition of the oropharyngeal microbiota found in the admission culture, either by absenting drug-sensitive microorganisms or by inserting other microorganisms, but these changes in the microbiota are independent of the use of antibiotics, since the samples were taken before surgical antibiotic prophylaxis and there is no record of antibiotics being administered to patients before surgery.

Regarding the type of change in the aerobic microbiota of the oropharynx, there were more changes due to the subtraction of a particular microorganism than the insertion, although these values are very close. The microorganisms found on admission and which were not found after 48 hours of hospitalization are suggestive of transient microbiota.

It can also be taken into account that secretion samples collected with a swab have low sensitivity, which can lead to the loss of some microorganisms present in the sample, but even with this possible interference, it was possible to show that there were changes in the oropharyngeal microbiota, important changes, as the results reveal the insertion of pathogenic microorganisms that are often related to hospital infection, although these microorganisms can colonize transiently without causing infection. Reducing the length of hospitalization could be one of the solutions to avoid acquiring microorganisms from the hospital environment. Collecting data from patients after they return to the outpatient clinic to check the surgical wound and other clinical information can help find out if there has been a surgical site infection, for example. Taking a new culture of the oropharyngeal secretion after this period can also help to check whether the microorganisms that were inserted are still present. In this way, it is possible to remove the doubt as to whether it was a transitory microbiota that was expelled over time or whether it really was an acquired microorganism that settled in the oropharynx.

CONCLUSION

The microorganisms identified in this study were: *Candida albicans, Candida tropicalis, Enterobacter aerogenes, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Micrococcus* spp, *Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Staphylococcus coagulase negative*, *Streptococcus viridans.*

There was a change in the oropharyngeal microbiota 48 hours after hospitalization in 64.64% of the individuals surveyed.

THANKS

I would like to thank Dr. Carlos N. Lehn, Dr. Thaís Guimarães, Dr. Fernando Danelon Leonhardt, Dr. Climério do Nascimento, Dr. Cídia Vasconcelos for their valuable advice and Dr. Judith Maria Vero for her help with the English language.

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