

## Communication

[Comunicação]

### Normal microbiota of the perialveolar region of incisors of rats

[Microbiota normal da região perialveolar de incisivos em ratos]

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Normal microbiota refers to the population of microorganisms inhabiting the skin and mucosa of healthy beings (Paster et al., 2001; Ye et al., 2003). It may benefit the host through microbial antagonism, thereby preventing the excessive growth of harmful microorganisms. Thus, the pathological process is triggered only when there is a disruption of the balance between normal microbiota and pathogenic microbes (Tortora et al., 2000). The oral cavity, for example, presents one of the most concentrated and varied population of microbes (Rosan and Lamont, 2000; Takarada et al., 2004). Koller et al. (2000a), in an experimental study in rats, found that Gram-positive cocci corresponded to the bacteria most commonly encountered in normal oral microbiota deposited on the teeth of these animals. Thus, these cocci accounted for 80% of the total and were distributed as follows: *Enterococcus*, 56%; *Streptococcus*, 14%; and *Staphylococcus*, 30%.

Despite the fact that rodents are the subject of several studies in dentistry, few reports are available on the normal microbiota of the oral cavity, in particular, on normal bacterial microbiota of the perialveolar region of incisors of rats.

Seventy-two male Wistar rats (*Rattus norvegicus albinus*), aging from 70 to 90 day-old, weighing between 280 and 330g, obtained from a bioterium, were used. The animals were kept in

propylene cages covered with zinc wire for alternating 12-hour periods of darkness and light, maintained on a solid diet<sup>1</sup> and *ad libitum* water, in accordance with the codes of ethics of the Brazilian College of Experimentation on Animals (Rivera, 1996) and the Institute of Laboratory Animal Resources Committee (NRC, 1992).

The animals were sedated by inhalation of sulphuric ether. Following sedation, the operating table was covered with a sterilized surgical drape and each rat was immobilized using a further surgical drape. Then, the animals were placed in the supine position to facilitate the opening of the mouth and separation of the lips in order to sample the bacterial microbiota from the perialveolar region of the incisors using swabs soaked in 40µl of a 0.9% NaCl sterile solution.

After collecting the microbiota, each swab was placed in a sterile tube containing 460µl of Brain Heart Infusion (BHI). Each sample was subsequently diluted by homogenizing the medium and drawing 100µl, which was then transferred to another sterile tube containing 900µl of BHI. These 1000µl were then homogenized, and with the aid of an 1µl calibrated loop, were spread onto Petri dishes containing blood-agar and Levine-agar for the isolation of Gram-positive and Gram-negative bacteria. The plaques were then incubated at 37°C in a bacteriological chamber for 48h and

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the colony-forming units (CFU) that developed were counted and their percentages calculated. Glass slides were prepared for performing Gram staining and to visualize the arrangements and shapes of the bacteria. In addition, the enterobacteria kit<sup>2</sup> was used for identification of Gram-negative bacteria, whereas Staphclin<sup>2</sup>, Novobiocin<sup>2</sup>, Optoquin<sup>2</sup>, Bile Esculin agar<sup>2</sup> and 6.5% NaCl were used for Gram-positive (Tomás et al., 2007).

Descriptive statistics was used for data analysis in order to obtain the percentage distributions, means and standard deviations (Zar, 1999).

Following analysis of the material collected from the 72 rats, the mean growth of the bacteria was  $1.4 \times 10^6 \pm 2.6 \times 10^5$  CFU/ml. In order of frequency, the aerobic bacteria found were as follows: *Bacillus* sp., *Staphylococcus aureus*, *Streptococcus viridans*, *Corynebacterium* sp., *Staphylococcus coagulase negative*, *Enterococcus* sp., *Staphylococcus saprophyticus*, *Escherichia coli*, *Klebsiella oxytoca*, *klebsiella pneumoniae* and *Serratia liquefaciens* (Table 1). Gram-positive and Gram-negative bacteria comprised 91.2% and 8.8% of all isolates, respectively.

Table 1. Bacteria isolated from the perialveolar region on incisors of 72 Wistar rats, listed in order of frequency.

Bacteria	Normal microbiota
<i>Bacillus</i> sp.	52-31.2%
<i>Staphylococcus aureus</i>	29-17.3%
<i>Streptococcus viridans</i>	29-17.3%
<i>Corynebacterium</i> sp.	28-16.8%
<i>Staphylococcus coagulase negative</i>	13- 7.8%
<i>Enterococcus</i> sp	11- 6.6%
<i>Staphylococcus saprophyticus</i>	5- 3.0%
<i>Escherichia coli</i> *	8-50.1%
<i>Klebsiella oxytoca</i> *	5-31.2%
<i>Klebsiella pneumoniae</i> *	2-12.5%
<i>Serratia liquefaciens</i> *	1- 6.2%

\*Gram-negative

With regard to the aerobic bacteria identified in the oral cavity, the findings are in agreement with those of other authors (Paster et al., 2001; Ye et al., 2003).

The microorganisms found within the oral cavity of domestic animals may be either commensal or pathogenic. Various studies have also revealed that

certain groups or genera of bacteria may be found in the oral microbiota of both humans and animals (Saphir and Carter, 1976). This is in agreement with Marcotte and Lavoie (1998) when they compared the normal microbiota present in humans, monkeys and rats and found the following microorganisms to be present in all three species: *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Corynebacterium*, *Actinomyces*, *Propionibacterium*, *Bacillus*, *Clostridium*, *Neisseria*, *Veillonella*, *Bacteroides*, *Fusobacterium*, *Spirochetes* and *Protozoa*.

In human beings, microorganisms colonize all the oral tissues irrespectively whether or not they are mineralized and their growth and survival depends on local microecological factors. Thus, it was possible to detect a predominance of Gram-positive cocci and bacilli on the surfaces of the teeth (Rodrigues and Newman, 2002), as observed in rats in the present study.

Chow (2000) reported that the proportion of the different species of bacteria varies in accordance to the presence of inflammatory and infectious processes. Genco (1999) demonstrated this occurrence in humans, pointing out that plaque cells associated with a healthy gingiva are mostly Gram-positive, whereas the filamentous forms and Gram-negative bacteria predominated when the gingiva is inflamed. Koller et al. (2000b) observed that the transition from periodontal health to the conditions known as gingivitis and periodontitis involved an increase in the microbiota growing on the tooth surfaces, particularly Gram-positive and Gram-negative cocci and bacilli. Rodrigues and Newman (2002) initially detected in such cases a Gram-positive aerobic microbiota, and later, a Gram-negative anaerobic. Socransky and Haffajee (2005) stated the fact that if this process continues to evolve, in addition to the coccoid form, other types of bacteria, such as fusobacteria, filaments, spirilla and spirochetes could be found.

Thus, a knowledge of this microbiota is extremely important, for it makes possible a comparison between normal microbiota and the inflammatory and infectious processes that take place in their oral cavity, in particular, gingivitis, periodontitis and alveolitis. The identification of normal microbiota from the perialveolar region of rats should therefore make a valuable contribution to future research in this area.

Keywords: rat, microbiota, aerobic bacteria, perialveolar region, alveolitis

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## RESUMO

Identificou-se a microbiota normal da região peri-alveolar de incisivos em 72 ratos Wistar, com 70-90 dias de idade e 280-330g de peso. As bactérias foram coletadas com suabes embebidos em solução salina. Do material depositado em tubo contendo 460µl de Brain Heart Infusion e diluído em 1:10, retirou-se 1µl para semeadura em placas de Petri. O crescimento médio foi  $1,4 \times 10^6 \pm 2,6 \times 10^5$  UFC/ml. Segundo a ordem das frequências, as bactérias encontradas foram: *Bacillus sp.*, *Staphylococcus aureus*, *Streptococcus viridans*, *Corynebacterium sp.*, *Staphylococcus coagulase negativa*, *Enterococcus sp.*, *Staphylococcus saprophyticus*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae* e *Serratia liquefaciens*. De todas as bactérias isoladas, as Gram-positivas e as Gram-negativas compreenderam 91,2% e 8,8%, respectivamente. Dentre as Gram-positivas, a mais frequente foi *Bacillus sp.* (31,2%) e a menos, *Staphylococcus saprophyticus* (3,0%). Quanto às bactérias Gram-negativas, a mais encontrada foi *Escherichia coli* (50,1%) e a menos, *Serratia liquefaciens* (6,2%).

Palavras-chave: rato, microbiota, bactéria aeróbia, região perialveolar, alveolite

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