Impact of oral surgery, with or without amoxicillin, on the oral microbiome, salivary flow and buffering capacity of saliva

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Abstract

Antibiotic prophylaxis is not usually recommended in the post-operative period due to tooth extraction operations. However, in more traditional clinical practices, the use of amoxicillin continues to represent the most influential factor in preventing bacterial infections. To evaluate the effectiveness of such an approach in the dental field, a phase IV, randomized, controlled, single-center double-masked clinical study was conducted, according to the split-mouth design. The investigation concerned only simple tooth extraction operations, in which patients were divided into two groups. Only one of the established groups was prescribed antibiotic therapy.
Thus, the evolution of the oral microbiome was evaluated through the count of Streptococcus mutants and Lactobacillus, the salivary flow, and the buffering power of the saliva before and after the extraction procedure.
Several units reported a significant change in bacterial load in the antibiotic-treated group, from baseline to seven days and fourteen days after surgery. In the same group, however, no substantial changes occurred in the salivary flow and the saliva's buffering capacity.
Given the high complexity of the salivary microbiome and its barrier functions for the human organism against attacks by pathogenic microbes, it is considered necessary to promote further studies aimed at investigating the mutations induced by antibiotic therapy, even if the results emerging from the most recent literature highlights the preventative role of prevention in post-extraction complications. However, given the low incidence of infections even in the absence of prevention, it becomes crucial to investigate how the risks of potential antibiotic resistance can compromise the benefits of antibiotic use

Keywords: Antibiotic prophylaxis in dentistry, Oral microbiology

Introduction

The trend of the presence of specific microbial populations within the oral microbiome can represent the prerequisite for the onset of dysbiosis in the organism (1). This condition is accentuated by the role of the oral cavity: the gateway to the digestive system, it represents a critical element in promoting the mixing of foods, active ingredients of drugs, microbiome, enzymes and digestive or salivary proteins (2) The imbalances resulting from the alteration of the oral microbiome thus seem to induce the disease condition of the human organism (3-5).
The abuse of antibiotic therapies in clinical practice has worsened the well-known phenomenon of bacterial resistance, so much so that it represents one of the main critical issues in public health. In Italy, in the absence of improving structural interventions, antibiotic-resistant bacterial infections are estimated to be up to 32% in 2030, with growth rates of two percentage points per decade. The OECD report "*Embracing a One*

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Health Framework to Fight Antimicrobial Resistance" (6) embodies this concern, stating that in 2023, one in five infections were resistant to traditional antibiotic therapies.

In the dental field, it is observed that amoxicillin is often prescribed in the preventive management of post-treatment infections despite the most recent research results highlighting its ineffectiveness in surgical applications if not accompanied by joint therapies. There is also the onset of local symptoms following tooth extractions such as dry alveolitis, feverish symptoms accompanied by swelling, and adverse enteric reactions closely linked to antibiotics. In the oral cavity, the further implications due to the clinical treatment of tooth extraction significantly impact the oral microbiome, requiring a correct prospective evaluation of the impact of amoxicillin on the patient's organism in the long term (7-8). Therefore, the objective of the present investigation is to evaluate the microbial changes in the oral cavity and the effects due to the use of the antibiotic in treated patients who require surgical extraction (9-10).

Materials and methods

Recruitment and grouping of subjects

Patients selected for simple extraction of dental elements were recruited after obtaining written informed consent. Subjects were advised to refrain from eating and performing oral hygiene procedures for at least two hours before sample collection.

Using the split-mouth design, 45 patients were enrolled, for a total of 90 teeth to be extracted. Two months passed between the intervention in one quadrant and the one in another to give the microbial flora the opportunity to re-stabilise. Among the selected patients, there were 24 women and 21 men. The average age for males was 33.3, and for females, 29.3. The patient variable, based on age, is summarized in Figure 1.

- The control group was treated with a placebo. The patients in this group will be used to evaluate whether the post-extraction complications are greater/lesser/the same as those of patients who received the antibiotic.
- The test group was treated with systemic antibiotics:
  - Augmentin 1g tablets (amoxicillin 875 mg + clavulanic acid 125 mg) every 12 hours.
  - For patients allergic to penicillin, 500 mg of Lincocin was used every 8 hours.

The standard prophylaxis regimen in adults includes 2g of amoxicillin administered orally for six days (4), so our patients will be provided with 2g of amoxicillin + clavulanic acid daily (1 gram every 12 hours) starting from the day before the extraction procedure dental treatment for 6 days.

Preliminary and follow-up visits

- First visit (T0) (data collection): during the first visit, the general state of the patient's oral cavity and the need to perform a dental extraction (not wisdom teeth) are assessed (11-16). The patient was informed of the possibility of participating in the study and its nature, and informed consent was drawn up if the patient agreed to participate. The operator then collected the patient's data, the medical history in which any allergies to drugs, heart disease or cardiovascular disorders, liver disease, kidney disease, infectious diseases, presence of diabetes mellitus, and cigarette smoking habits were investigated. If it was decided to continue with the study, the patient underwent salivary collection

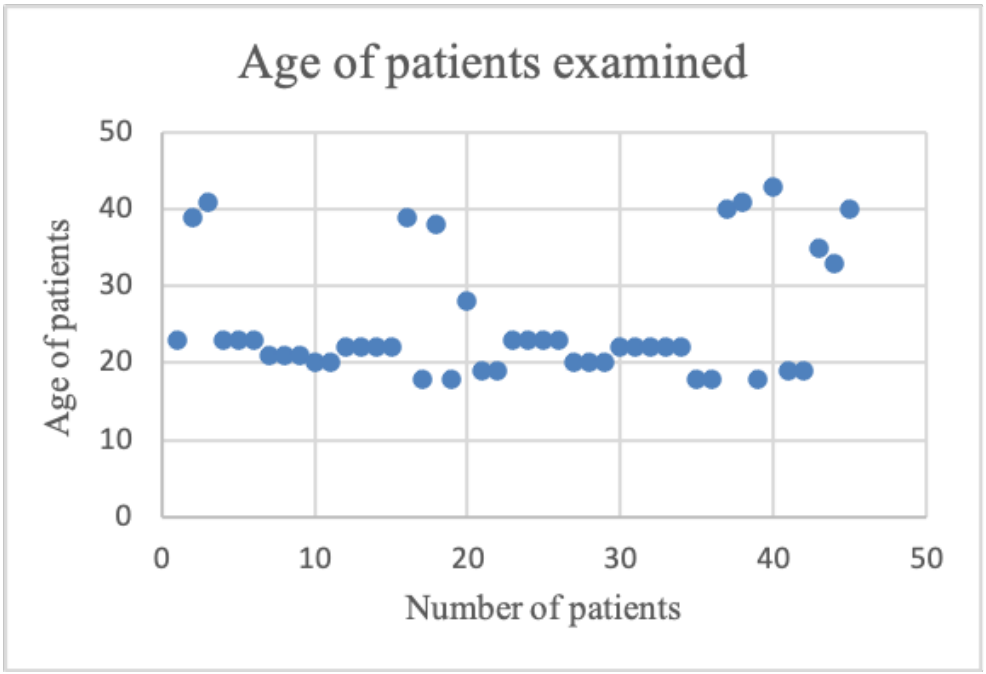

Figure 1. Age of patients examined

and bacteriological analysis. Once they returned home, they began the therapy provided to them (antibiotic or placebo).

- Second visit (T1) (tooth extraction): during the second visit, the extraction was carried out. Some information was collected on the tooth that needed to be extracted and the reason for its avulsion (root residue, failure of root canal therapy, periodontal disease, destructive caries, etc.). At this point, the extraction was performed after local anesthesia by injection (Mepivacaine hydrochloride + adrenaline 1:100,000 or without adrenaline in patients with cardiac arrhythmia)(17-18) The post-extraction socket was treated in the simplest way possible: only compression was performed with sterile gauze to stop the bleeding. All subjects were instructed with the exact post-operative conduct instructions: do not lie down for the first few hours following the extraction, avoid hot and harrowing foods, do not rinse for the first 24 hours to prevent the risk of dissolving the clot and tightening a gauze pad for 30 minutes if there was bleeding, apply ice to the external skin to reduce post-operative edema (19-22).

- Third visit (T2) (7 days after extraction): on the third visit, the investigator evaluated the variation in the bacterial load through the count of mutans streptococcus and lactobacilli, the salivary flow and the following parameters: edema; pain, assessed with the VAS scale; suppuration; fever; presence of alveolitis; generic post-extraction complications. The patient was also asked if the extraction had affected his daily activities. This was assessed through the following parameters: difficulty chewing, assessed with the VAS scale; difficulty speaking, continually assessed with the VAS scale; difficulty in carrying out regular oral hygiene practices, assessed with the VAS scale; alterations to the daily routine, evaluated through the loss of working and/or study days. Furthermore, the table given to the patient was assessed to note the analgesic/anti-inflammatory drugs taken in the first 7 days after the extraction in case he needed them. It was then necessary to evaluate the adverse effects resulting from the antibiotic. For this purpose, 2 forms were used which report the signs and symptoms presented by the patients, together with their frequency and intensity: abdominal pain, abdominal distension, changes in the bowel movement, characteristics of the bowel movement, nausea, vomiting, acid reflux, retrosternal heartburn, gastric pain, intestinal pain, gastric distension, intestinal distension, constipation, diarrhoea, loss of appetite. (23-26)

- Fourth visit (T3) (14 days after the extraction): the variation in the bacterial load was reconsidered through the count of mutans streptococcus and lactobacilli and the salivary flow.

Patient compliance with antibiotic treatment was confirmed by checking antibiotic tablet strips at recall appointments.

Saliva collection, DNA extraction, sequencing, and data analysis

1. Each subject was given a saliva-stimulating paraffin tablet, which had to be chewed for 30 seconds, and then the saliva was produced to exclude any contamination. The paraffin tablet was administered again and chewed for 5 minutes, collecting the saliva gradually produced in a graduated glass. Through this procedure, the milliliters of saliva collected in the set minutes and the salivary flow, i.e., the quantity of saliva produced in a given period (5 minutes), were calculated.
2. Using the CRT® buffer, the saliva's buffering power was calculated and evaluated in three values: low, medium, and high.
3. Using the CRT® bacteria, the count of Lactobacilli and Streptococcus mutans was carried out as follows: From the graduated glass, previously filled by the patient, a certain quantity of saliva was taken using pipettes, sufficient to completely wet the agar culture media.
4. A $NAHCO_3$ tablet was inserted into the culture medium container to stimulate bacterial growth.
5. Everything was placed in an incubator at 35-37 °C for 48 hours.
6. Subsequently, each patient was given the antibiotic or placebo, with the conditions of use listed previously.
7. Seven days after the operation, during which the antibiotic or placebo was taken, the patients underwent a second check with CRT® buffer and CRT® bacteria.

Results and discussion

The tables below show the results of both the control group and the test group, the day before (T0), seven days after (T1), and fourteen days after (T2) the operation, for Streptococcus mutans and Lactobacilli.
The numbers 0 and 1 in the tables below indicate that the bacterial colony count was significantly lower or higher than the value of 10^5 colony forming units.
As can be seen by comparing the data collected, it is highlighted that although the buffer capacity has not changed, the bacterial count has significantly reduced. In fact, at the beginning of the data collection, both the Streptococcus Mutans and Lactobacillus colonies were much higher in number compared to the analyzes carried out after the treatment.

Conclusions

This study shows how antibiotic prophylaxis can reduce post-extraction complications, but given the low rate of infections even in the absence of prevention, it is essential to consider whether the risks outweigh the resulting benefits.

Table 1. Bacterial counts of Streptococcus Mutans and Lactobacillus, before (T0), 7 days after (T1) and 14 days after (T2) surgery.

Streptococco Mutans

Gruppo controllo				Gruppo test			
N°	T0 ctr	T1 ctr	T2 ctr	N°	T0 tst	T1 tst	T2 tst
1	0	0	0	1	0	0	0
2	0	0	0	2	0	0	0
3	1	1	1	3	1	0	0
4	0	0	0	4	0	0	0
5	0	0	0	5	0	0	0
6	1	1	1	6	1	0	0
7	0	0	0	7	0	0	0
8	0	0	0	8	0	0	0
9	1	1	1	9	1	1	0
10	0	0	0	10	0	0	0
11	0	0	0	11	0	0	0
12	0	0	0	12	0	0	0
13	0	0	0	13	0	0	0
14	0	0	0	14	0	0	0
15	1	1	1	15	1	0	1
16	0	0	0	16	0	0	0
17	0	0	0	17	0	0	1
18	0	0	0	18	0	0	1
19	0	0	0	19	0	0	0
20	0	0	0	20	0	0	0
21	0	0	0	21	0	0	0
22	0	0	0	22	0	0	0
23	1	1	1	23	1	0	0
24	0	0	0	24	0	0	0
25	0	0	0	25	0	0	0
26	0	0	0	26	0	0	0
27	0	0	0	27	0	0	0
28	0	0	0	28	0	0	0
29	1	1	1	29	1	0	0
30	0	0	0	30	0	0	0
31	0	0	0	31	0	0	0
32	0	0	0	32	0	0	0
33	0	0	0	33	0	0	0
34	0	0	0	34	0	0	0
35	1	1	1	35	1	0	0
36	0	0	0	36	0	0	0
37	0	0	0	37	0	0	0
38	0	0	0	38	0	0	0
39	0	0	0	39	0	0	0
40	0	0	0	40	0	0	0
41	1	1	1	41	1	0	0
42	0	0	0	42	0	0	1
43	0	0	0	43	0	0	0
44	1	1	1	44	1	0	0
45	1	1	1	45	1	0	1

Lactobacillus

Gruppo controllo				Gruppo test			
N°	T0 ctr	T1 ctr	T2 ctr	N°	T0 tst	T1 tst	T2 tst
1	1	0	0	1	1	0	0
2	0	0	0	2	0	0	0
3	0	0	0	3	0	0	0
4	0	0	0	4	0	0	1
5	0	0	0	5	0	0	0
6	0	0	0	6	0	0	0
7	0	0	0	7	0	0	0
8	0	0	0	8	0	0	1
9	1	1	1	9	1	0	1
10	0	0	0	10	0	0	0
11	0	0	0	11	0	0	0
12	0	0	0	12	0	0	0
13	0	0	0	13	0	0	1
14	1	1	1	14	1	0	1
15	0	0	0	15	0	0	1
16	0	0	0	16	0	0	0
17	0	0	0	17	0	0	1
18	0	0	0	18	0	0	1
19	0	0	0	19	0	0	0
20	1	1	1	20	1	0	0
21	0	0	0	21	0	0	0
22	0	0	0	22	0	0	0
23	1	1	1	23	1	0	0
24	0	0	0	24	0	0	0
25	0	0	0	25	0	0	0
26	1	1	1	26	1	0	0
27	0	0	0	27	0	0	0
28	0	0	0	28	0	0	0
29	1	1	1	29	1	0	0
30	0	0	0	39	0	0	0
31	0	0	0	31	0	0	0
32	0	0	0	32	0	0	0
33	0	0	0	33	0	0	0
34	0	0	0	34	0	0	0
35	1	1	1	35	1	0	0
36	0	0	0	36	0	0	0
37	0	0	0	37	0	0	0
38	1	1	1	38	1	0	0
39	0	0	0	39	0	0	0
40	0	0	0	40	0	0	0
41	1	1	1	41	1	0	0
42	0	0	0	42	0	0	1
43	0	0	0	43	0	0	0
44	1	1	1	44	1	0	0
45	1	1	1	45	1	0	1

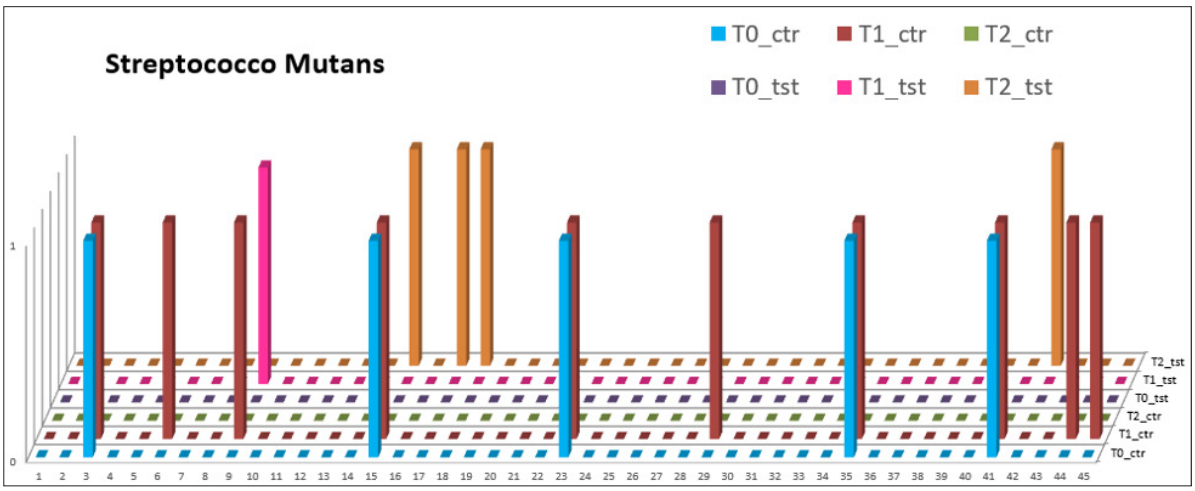

Figure 2. Example image of Streptocco Mutans bacterial count

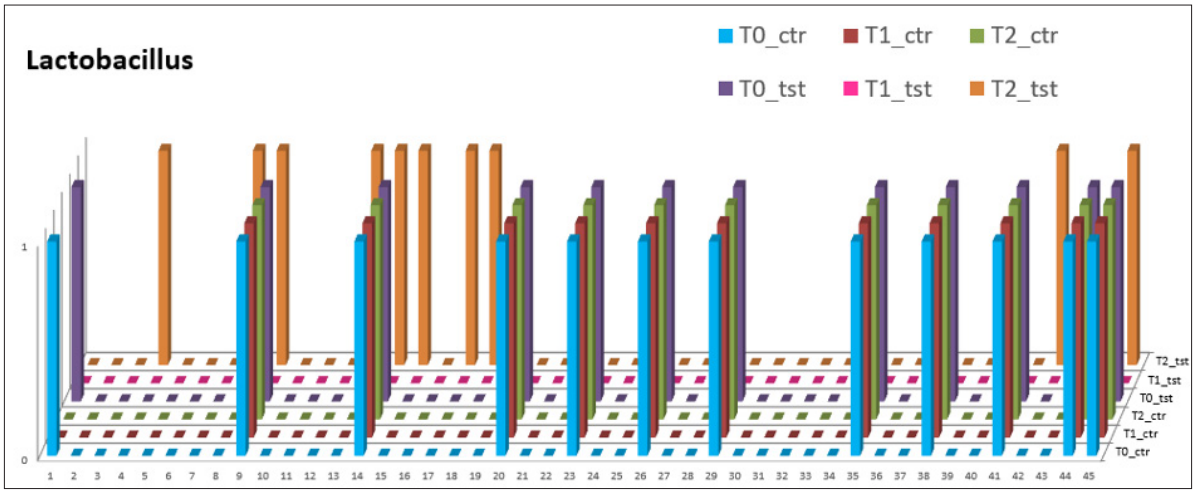

Figure 3. Example image of Lactobacillus bacterial count

Table 2. The buffering power of saliva in both groups was also evaluated

Potere tampone della saliva							
Gruppo Test				Gruppo Controllo			
N°	T0	T1	T2	N°	T0	T1	T2
1		basso		1		basso	
2		medio/basso		2		medio/basso	
3		basso		3		basso	
4		medio		4		medio	
5		basso		5		basso	
6		medio		6		medio	
7		medio		7		medio	
8		medio/alto		8		medio/alto	
9		basso		9		basso	
10		alto		10		alto	
11		medio		11		medio	
12		medio/basso		12		medio/basso	
13		medio		13		medio	
14		basso		14		basso	
15		basso		15		basso	
16		medio		16		medio	
17		medio		17		medio	
18		medio/alto		18		medio/alto	
19		alto		19		alto	
20		basso		20		basso	
21		basso		21		basso	
22		medio/basso		22		medio/basso	
23		basso		23		basso	
24		medio		24		medio	
25		basso		25		basso	
26		medio		26		medio	
27		medio/alto		27		medio/alto	
28		basso		28		basso	
29		alto		29		alto	
30		medio		30		medio	
31		medio/basso		31		medio/basso	
32		medio		32		medio	
33		basso		33		basso	
34		basso		34		basso	
35		medio		35		medio	
36		medio		36		medio	
37		medio/alto		37		medio/alto	
38		alto		38		alto	
39		basso		39		basso	
40		basso		40		basso	
41		medio		41		medio	
42		medio		42		medio	
43		medio/alto		43		medio/alto	

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