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Efeito da penicilina G a cada três semanas sobre o surgimento de Streptococcus viridans resistentes à penicilina na microflora oral

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Original Article

Effect of Penicillin G Every Three Weeks on Oral Microflora by Penicillin Resistant Viridans Streptococci

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Abstract

Background: **Benzathine penicillin G every 3 weeks is the standard protocol for secondary prophylaxis for recurrent rheumatic fever.**

Objective: **Assess the effect of Benzathine penicillin G on *Streptococcus sanguinis* and *Streptococcus oralis* in patients with cardiac valvular disease due to rheumatic fever receiving secondary prophylaxis.**

Methods: **Oral streptococci were evaluated before (baseline) and 7 days (day 7) after Benzathine penicillin G in 100 patients receiving routine secondary rheumatic fever prophylaxis. Saliva samples were evaluated for colony count and presence of S. sanguinis and S. oralis. Chewing-stimulated saliva samples were serially diluted and plated onto both nonselective and selective 5% sheep blood agar containing penicillin G. The species were identified using conventional biochemical tests. Minimal inhibitory concentrations were determined with the Etest.**

Results: **No statistical differences were found in the presence of S. sanguinis comparing baseline and day 7 (p = 0.62). However, the existing number of positive cultures of S. oralis on day 7 after Benzathine penicillin G presented a significant increase compared to baseline (p = 0.04). No statistical difference was found between baseline and day 7 concerning the number of S. sanguinis or S. oralis CFU/mL and median minimal inhibitory concentrations.**

Conclusion: **This study showed that Benzathine penicillin G every 3 weeks did not change the colonization by S. sanguinis, but increased colonization of S. oralis on day 7 of administration. Therefore, susceptibility of *Streptococcus sanguinis* and *Streptococcus oralis* to penicillin G was not modified during the penicillin G routine secondary rheumatic fever prophylaxis. (Arq Bras Cardiol 2012;98(5):452-458)**

Keywords: **Penicilin G benzathine/therapeutic use; rheumatic fever; heart valve diseases; mouth; *Viridans streptococcus; Streptococcus oralis*.**

Introduction

Rheumatic fever (RF) is the leading cause of valvular heart disease in developing countries. RF causes significant morbidity and mortality, causing 90% of cardiac surgeries in children and over 30% of cardiac surgeries in adults. Secondary prophylaxis of 1,200,000 U benzathine penicillin G (BPG) every 3 weeks is the standard regimen for the prevention of recurrent rheumatic fever in developing countries. Valvular sequelae is the most dreadful consequence of acute rheumatic fever, and such cardiac lesions can also predispose a patient to infective endocarditis (IE), a morbid disease that worsens the prognosis of these patients[1-4].

In spite of the widely recommended secondary prophylaxis of RF with BPG every 3 weeks[2,5,6], very few studies have assessed the antibiotic susceptibility and frequency of Viridans *Streptococci* in the oral flora of patients receiving secondary prophylaxis after RF with BPG. Also, none of the studies have assessed this issue related to *S. sanguinis* and *S. oralis*, which are predominant species recovered in IE[7,8].

BPG prophylaxis protects valvular heart disease patients of new RF recurrences. However, no study has evaluated the oral flora with an expressive casuistic and specificity for these pathogens.

Therefore, the aim of this study is to evaluate if there is an association between BPG prophylaxis and the colonization of oral cavity by penicillin resistant Viridans *Streptococci*.

Methods

Cohort

A hundred patients were selected and evaluated in 2 periods:

BPG baseline and day 7 – One hundred RF patients previously receiving a secondary prophylaxis regimen with benzathine penicillin G 1,200,000 IU for at least 6 months before study admission. Patients with rheumatic activity or patients with diagnosis of infection were excluded.

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All patients were assessed clinically, and they underwent oral cavity inspection to avoid admission of patients with acute systemic or oral infection.

This study was approved by the institutional ethics committee, and all study patients gave informed consent.

Sample collection, transport, and culture

Samples were collected at baseline (100 samples) and day 7 (100 samples) after BPG prophylactic dosage. Saliva samples were obtained from patients who chewed paraffin tablets[9] and were collected into a sterile disposable plastic vial. Approximately 1 mL was transferred to a vial containing 5.7 mL of Göteborg viability medium, anaerobically prepared and sterilized (VMGA II S)[10]. Samples in transport medium were maintained at 2ºC to 8ºC before plating. Saliva samples were serially diluted tenfold in phosphate-buffered solution pH 7.2 before plating onto Columbia nalidixic agar plus 5% sheep blood (CNASB), CNASB plus penicillin G (0.25 μg/mL)[11], and amphotericin B (0.5 μg/mL)[12-15]. Media were incubated at 35ºC in 5% CO_2 for 72 h and then analyzed for colony count for each colonial morphotypes.

Species identification

Each colony morphotype was subcultivated onto tryptic soy agar plus 5% sheep blood (SBA) and tested for catalase and Gram stain. Catalase negative Gram-positive cocci were subsequently evaluated for the following characteristics/substrate utilization: hemolysis, arginine, urea, L-pyrrolidonyl-β-naphthylamide and esculin hydrolysis, β-N-acetyl glycosidase, α-D-glycosidase, β-N-acetyl galactosidase, optochin susceptibility, Voges-Proskauer test, and acid production from inulin, mannitol, raffinose, and melibiose, as recommended[16].

Susceptibility testing

Minimal inhibitory concentration (MIC) for penicillin G was determined using the Etest as recommended by the manufacturer (AB Biodisk, Solna, Sweden). Results were interpreted as recommended by the Clinical and Laboratory Standards Institute (CLSI)[11].

Statistical analysis

The Logistic Regression model was used to check the group's association with the occurrence of *S. sanguinis* and *S. oralis* with age and sex corrections.

The qualitative analysis was performed using the Mc Nemar nonparametric test.

Quantitative analysis was performed with the use do the Wilcoxon nonparametric test.

Statistical significance of 5% was applied for all tests.

Results

The group studied was composed of 100 patients, 38 males, aged 10 to 53 years old (26.5 ± 8 years).

In the group, 54 patients had mitral regurgitation, 27 had aortic regurgitation, 25 had mitral stenosis, and 14 had aortic stenosis. Eight patients had biological mitral prosthesis, 3 had biological aortic prosthesis, 2 had mechanical mitral prosthesis, and 1 had mechanical aortic prosthesis.

There were no differences comparing the number of positive cultures existed for *S. sanguinis* at baseline and day 7 after BPG (p = 0.62, Table 1). However, the existing number of positive cultures for *S. oralis* at day 7 after BPG presented a significant increase compared to baseline (p = 0.04, Table 1).

The assessments of the CFU/mL number in saliva of the patients at baseline and day 7 after BPG were subdivided into *S. sanguinis* and *S.* oralis.

The CFU/mL values in saliva for *S. sanguinis* and *S. oralis* did not differ between baseline and day 7 after BPG (p = 0.68 and p = 0.80 respectively; Figure 1).

The minimal inhibitory concentrations for penicillin G values were subdivided into *S. sanguinis* and *S. oralis*. No statistical difference was found between baseline and day 7 after BPG concerning the MIC of *S. sanguinis* and *S. oralis* (p = NS; Figure 2).

Table 2 shows data as MIC_{50} and MIC_{90}, which represents the minimal inhibitory concentrations for penicillin G to inhibit 50% and 90%, respectively, of the susceptible *Streptococcus sanguinis* and *Streptococcus oralis* to penicillin G.

Discussion

This group of patients with valvular heart disease is predisposed to infective endocarditis. Infective endocarditis results from bacteremia, often related to oral infectious focuses. *Viridans streptococci* are the predominant group recovered in IE, particularly *Streptococcus sanguinis* and *Streptococcus oralis*. The effect of chronic BPG has not been studied with specificity to these pathogens yet.[22,23]

The study cohort had a prevalence of females (62/100), possibly due to the higher general occurrence of RF in women[17]. Despite the gender heterogeneity, it did not represent a bias because no statistical difference existed (p = NS) between the occurrence of *S. sanguinis* an *S.oralis* in relation to the patients age and sex when we applied the logistic regression model. Furthermore, no statistical difference existed among the rate of tooth cavities, loss of teeth, or teeth with fillings between sexes in the study population[18]. All patients had their oral cavities inspected according to the criteria of the World Health Organization[19] to exclude the presence of oral infections, which could affect the oral microbiota[16,20-22].

S. sanguinis and *S. oralis* are common commensals of the oral cavity present in the initial formation of dental bacterial plaque, representing nearly 80% *Streptococcus* in this phase[20-23]. The BPG 1,200,000 IU IM dose exercises as a bactericide effect on the *Streptococcus,* sensitive in the active multiplication phase, which, hypothetically, would cause a decrease in these microorganisms in the oral cavity in this group of patients.

S. sanguinis was also observed in both periods in a limited number of patients, similarly to that observed by Bilavsky et al[7]. No difference (*P*=0.62) of its presence was noticed in the study groups. This showed that BPG did not interfere with the species growth. However, other studies reported a greater number of samples of *S. sanguinis*[20,21], perhaps due to the use of classic methodology for *Streptococcus* identification (with the use of commercially available identification kits in conjunction with

Table 1 – **Positive cultures for *Streptococcus sanguinis* and *Streptococcus oralis***

Groups	*Streptococcus sanguinis*	*Streptococcus oralis*
Baseline*	11	87
Day 7†	9	95‡

**Patients before benzathine penicillin G dosage; †Patients under benzathine penicillin G 7 day effect; ‡p = 0.04.*

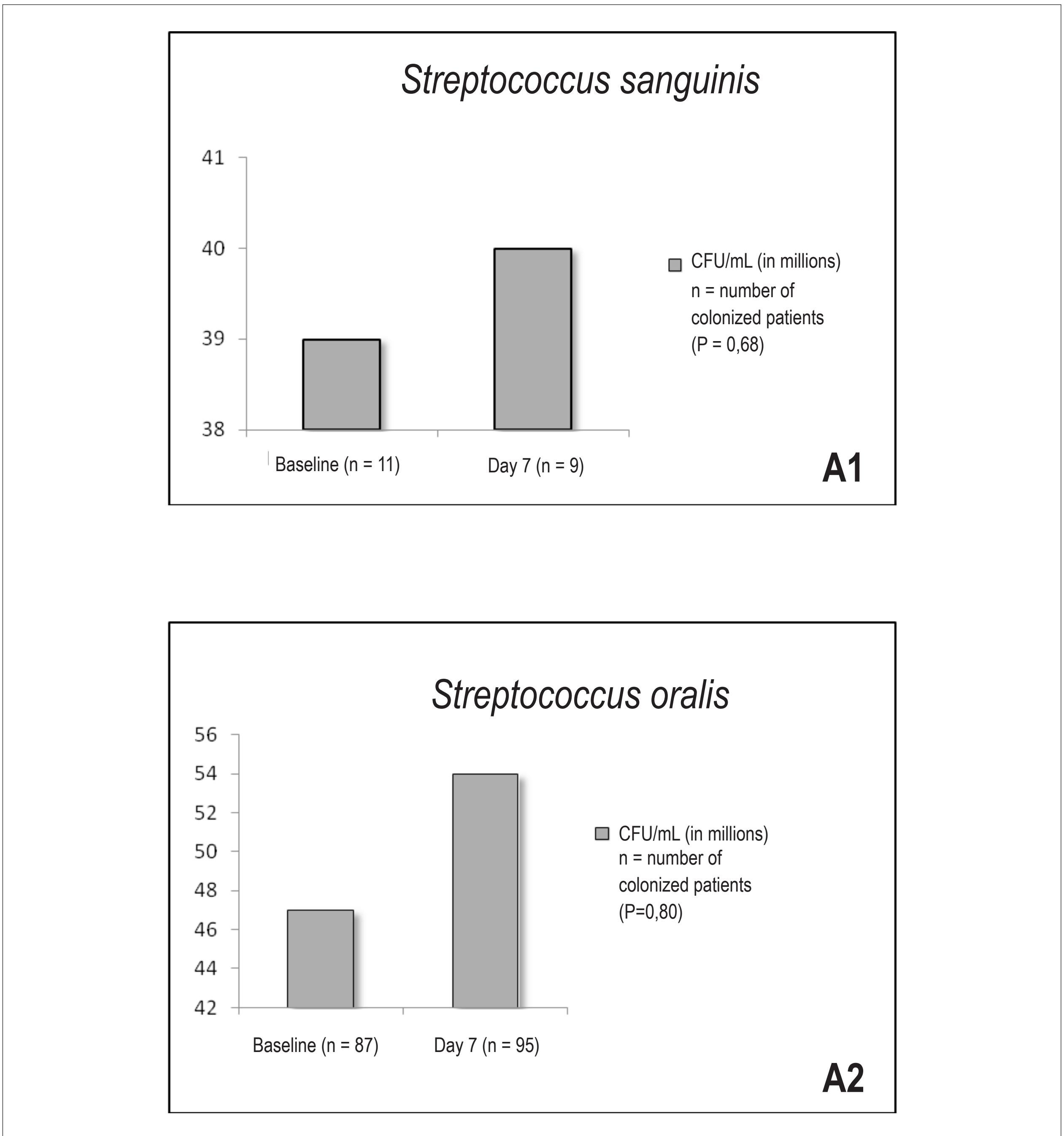

Figure 1 – *A1 - Distribution of CFU/mL values in saliva in patients colonized by S. sanguinis; A2 - distribution of CFU/mL values in saliva in patients colonized by S. oralis.*

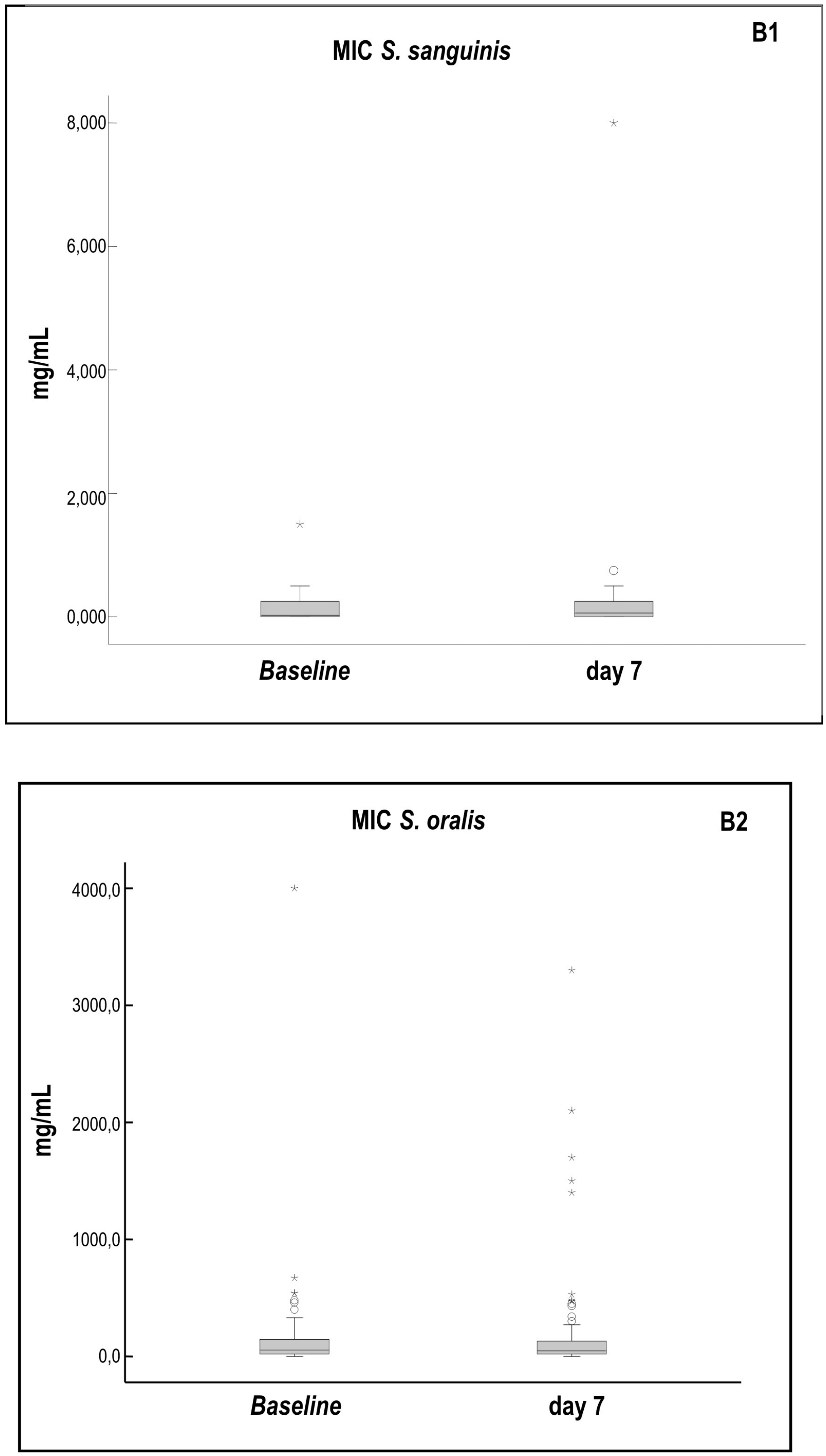

Figure 2 – *B1 - distribution of MIC values in µg/mL for S. sanguinis; B2 - distribution of MIC values in µg/mL for S. oralis*

Table 2 – MIC_{50} and MIC_{90} (µg/mL) and group distribution according to *Streptococcus sanguinis* and *Streptococcus oralis* susceptibility to penicillin

	Baseline‡		Day 7§	
	S. sanguinis	S. oralis	S. sanguinis	*S. oralis*
MIC_{50}*	0.190	0.250	0.250	0.250
MIC_{90}†	0.380	1.000	0.500	1.000
Sensitive	45.5% (5/11)	44.8% (39/87)	33.3% (3/9)	41.1% (39/95)
Intermediary resistance	45.5% (5/11)	55.2% (48/87)	66.7% (6/9)	58.9% (56/95)
High level resistance	9% (1/11)	-	-	-

MIC_{50} – minimal inhibitory concentration for penicillin G to inhibit 50% of the susceptible Streptococcus sanguinis and Streptococcus oralis. †MIC_{90} – minimal inhibitory concentration for penicillin G to inhibit 90% of the susceptible Streptococcus sanguinis and Streptococcus oralis. ‡Patients before benzathine penicillin G dosage. §Patients under benzathine penicillin G 7 day effect. P = NS.

standard screening tests for the genre). Nevertheless, in this study the methodology described by Ruoff et al[16] was adopted, performing biochemical tests with the addition of 3 fluorogenic substrates, increasing the specificity in the identification of the species. Although we have used the best methodology available for the identification of these species, it is known that there are some limitations, since genetic sequencing would certainly be the best method to be applied. However, this would be financially impractical due to the sample size, probably leading to no difference in the result of the study.

In regard to obtaining the samples through stimulated saliva and not from the dental plaque, this was due to an overall knowledge that stimulated saliva is a better collection method for its homogeneity and logistics compared to the collection of dental plaque samples[24].

As for the *S. oralis*, we noticed a significantly higher number of these microorganisms in the BPG-day 7 vs. baseline (p = 0.04, Table 1). The saliva sample collected from BPG- day 7 occurred during the time of the greatest amount of serum drug concentration according to Decourt et al[25]. This is evidence of the lack of BPG influence on the most prevalent microorganism in the bacterial plaque and one of the main Infectious Endocarditis etiological agents[20,21,26].

In this study, we found no statistical difference concerning saliva UFC/mL numbers (Figure 1, p = NS) and MICs (MIC_{50} and MIC_{90}) of *S. sanguinis* and *S. oralis* (Table 2).

The chronic use of benzathine penicillin G in the study group did not significantly alter *S. sanguinis* and *S. oralis* susceptibility to penicillin G, as seen previously[7]. It is interesting to note the occurrence of an increase in the resistance, or MIC values, in Viridans *Streptococcus* and, therefore, an increase in the number of strains resistant to antibiotics isolated into positive hemocultures for IE. However, these patients were receiving oral antibiotic therapy, and MIC intervals differ from those standardized by CLSI for penicillin G[7,27,28].

Our findings regarding the identification of species corroborate the results of Bilavsky et al[7]. However, we disagree with the increased resistance to penicillin G of Viridans *Streptococcus*.

This study contributed to awake a inquiry regarding the clinical treatment of these patients. Although the American Heart Association (AHA) no longer recommends antibiotic prophylaxis prior to procedures that cause bacteremia in these patients, we believe that further studies are required aimed at the Brazilian population to know our reality, and then adapt it to changes suggested by the AHA. While we are awaiting these data, it would be prudent to continue to implement the recommendations of the 1997 AHA guidelines, in which prophylaxis for infective endocarditis is recommended for patients with rheumatic heart disease prior to procedures that cause bacteremia in the genitourinary tract, respiratory tract and the stomatognathic system.

Our study also contributes to the interface between medicine and dentistry, because it shows that under the prolonged action of PGB, there is no decrease of the main microorganisms involved in the etiology of infective endocarditis and, therefore, antibiotic prophylaxis prior to procedures that cause bacteremia may be necessary.

Hence, additional studies are necessary to verify the need to establish a special routine so that these patients can undergo dental procedures that could cause bacteremia on the 7th day following the administration of BPG.

This study showed that BPG every 3 weeks did not change the colonization by *S. sanguinis*; but BPG increased *S. oralis* colonization on day 7 following its administration and, finally, susceptibility of *Streptococcus sanguinis* and *Streptococcus oralis* to penicillin G was not altered during the penicillin G cycle.

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Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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