Antibacterial Effectiveness of Platelet Products (PRP, PPP, PCP, and PFC) on *M. catarrhalis* and *S. aureus*, a Causative Agent of Acute and Chronic Sinusitis, Respectively

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Abstract

Objective: The antibacterial characteristics of the platelet products against various microorganisms have been emphasized. Here we evaluated the antibacterial effectiveness of platelet rich plasma (PRP), platelet poor plasma (PPP), platelet-concentrated plasma (PCP), and noncoagulating platelet-derived factor concentrate (PFC) on *Moraxella catarrhalis* and *Staphylococcus aureus* in *in vitro* conditions.

Material and Methods: Platelet rich plasma (PRP), PPP, PCP, and PFC were prepared. The standard strains of *M. catarrhalis* and *S. aureus* were mixed with the platelet solutions *in vitro*. Following incubation, the growing colonies were counted. This was repeated thrice.

Results: The efficacies of PRP and PPP were found to be present against both bacteria in all dilutions. While PCP was effective only in the 1:1 dilution. No effectiveness of PFC was identified in any of the dilutions.

Conclusion: The determination of significant antibacterial effects of PRP and PPP against *S. aureus* and *M. catarrhalis* indicate that they can be used in addition to the management of acute and chronic sinusitis.

Keywords: Antibacterial, platelet, *staphylococcusaureus, moraxellacatarrhalis*

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INTRODUCTION

The antibacterial characteristics of the platelet rich plasma (PRP) against various microorganisms have been emphasized in numerous studies (1-3). For example, while the antibacterial efficacy of PRP has been determined against *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, and *Klebsiella pneumoniae,* PRP has been identified to be ineffective against *Pseudomonas aeruginosa, Enterobacter cloacae, Bacillus cereus*, and *Bacillus subtilis* (1, 2, 4-7). However, which component of the platelets provides the antibacterial effect has not been clearly identified to date. Some platelet proteins, such as alpha granule components and complement-binding proteins, have been suggested to be effective (8). The direct interaction of the platelet with the microorganism, release of myeloperoxidase, antibody-independent cellular toxicity, and antigen-specific immune response were stated to be responsible for this effect (9). The efficacy of the peptides released from the platelets against gram-negative, gram-positive, and fungal pathogens has been reported (10, 11). Moreover, in a study conducted in *S. aureus,* the metabolic activity for biofilm formation was reportedly reduced at a rate of 7%-38% and a synergistic effect was present with beta-lactam antibiotics (12).

S. aureus is a gram-positive bacteria detected at a rate of 50% in chronic sinusitis (13, 14). *S. aureus* shows its effect by changing its phenotype after entering the cell, leading to gene regulation and virulence changes (15). The studies have particularly shown that the colonization of *S. aureus* increases in chronic sinusitis with polyp (16). In a meta-analysis study conducted by Payne et al. (17), *S. aureus* was found at a rate of 9.7% higher than *M. catarrhalis* in acute bacterial rhinosinusitis.

M. catarrhalis is a gram-negative, aerobic, oxidase-positive diplococcus bacteria present in the upper respiratory tract, particularly in children. It shows its effect by releasing endotoxin. It may lead to resistance by changing its membrane attack complex. A high proportion of beta-lactamase positivity is present; therefore, it is resistant to ampicillin (18).

In the present study, we evaluated the antibacterial effectiveness of PRP, platelet poor plasma (PPP), platelet-concentrated plasma (PCP), and non-coagulating platelet-derived factor concentrate (PFC) on *M. catarrhalis,* which is one of the causative agents of acute sinusitis, and on *S. aureus,* which is one of the causative agents of acute or chronic sinusitis, in in-vitro conditions.

MATERIAL AND METHODS

The study was conducted between October 2015 and December 2015 following the approval of the Adnan Menderes University Ethics Committee for Non-Interventional Clinical Studies and with the Support of Adnan Menderes University Commission for Scientific Research Projects TPF-16002.

Platelet rich plasma (PRP), PPP, PCP, and PFC were prepared according to the protocol suggested by Araki et al. (19). Six tubes of whole blood were collected from a healthy volunteer. Following centrifugation of the whole blood placed into the 15 mL ethylenediaminetetraacetic acid (EDTA) tubes at 230 g for 10 min, PRP was obtained by collecting the plasma separated in the uppermost layer. PPP and platelet cluster were obtained by centrifuging this PRP at 2330 g for 10 min. The PPP portion was separated. The remaining platelet cluster (1/10 volume) was recentrifuged and activated by calcium chloride and PCP was obtained. For obtaining PFC, the PPP portion of the product obtained by centrifugation of PRP was removed and replaced by phosphate-buffered saline in a 1/10 volume; it was recentrifuged and activated by thrombin.

In an aseptic environment, following the calibration of the standard strains of *M. catarrhalis* and *S. aureus* (*S. aureus* ATCC 25923, *Moraxella catarrhalis* ATCC 49143) with the 0.5 McFarland turbidity setting in the liquid medium, the final concentration being adjusted to 10^5 bacteria. The cultures were mixed with the platelet solutions (PRP, PPP, PCP, and PFC) in the tubes at concentrations 1:1, 1:2, 1:4, and 1:8 and incubated at 35°C for 24 h. Subsequently, 10 µL of each sample was taken, diluted with the liquid medium as 1/100 and 1/1000, and 10 µL was inoculated in 5% sheep blood medium and kept in the incubator at 35°C for 24 h. Following incubation, the growing colonies were counted; the colony-forming units (CFUs) were calculated by multiplying the colony number in 1/100 dilutions by 100 and in 1/1000 dilutions by 1000. The antibacterial effect was defined as the number of CFU. This assay was repeated thrice at various times.

Statistical Analysis

Because the variables had extreme values in the data set, the analyses were carried out using the non-parametric tests. For comparison of variables between two independent groups, the Mann-Whitney U test was used. For comparison of the dependent groups, the Friedman Two-Way Analysis of Variance was used. The descriptive statistical results were expressed as median (25^{th}-75^{th} percentiles). $p<0.05$ was considered statistically significant. Post-hoc tests were done with Bonferroni correction.

RESULTS

The efficacies of PRP, PPP, and PFC in 1:1, 1:2, 1:4, and 1:8 dilutions were studied on *S. aureus* and *M. catarrhalis.* Efficacies of PRP and PPP were found to be present against both bacteria in all dilutions (Figure 1 and 2). While PRP was found to be more efficient in 1:8 dilution on *S. aureus* compared to PPP, the efficacies of PRP and PPP on *M. catarrhalis* in the 1:8 dilution were determined to be less compared to the other dilutions. While PCP was as effective as PRP and PPP in the 1:1 dilution, no efficacy of PCP was identified in the other dilutions. No effectiveness of PFP was identified in any of the dilutions (Table 1 and 2).

When the efficacies against *S. aureus* and *M. catarrhalis* were compared, no differences were found in the 1:1 and 1:2 dilutions concerning PRP and PPP ($p>0.05$ and $p>0.05$). However, in the 1:4 dilution, the efficacy against *M. catarrhalis* was found to be significantly lower for PRP ($p=0,009$; Figure 3, Table 3).

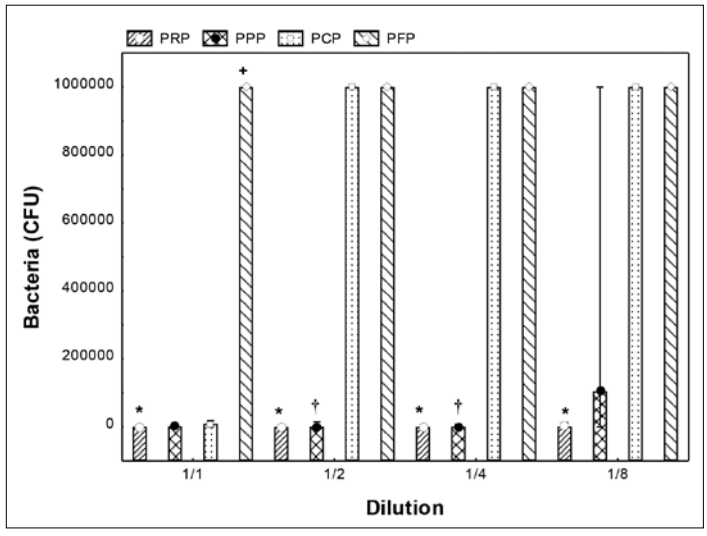

Figure 1. Comparison of the antibacterial effect of S. aureus between all solutions and dilutions
*: PRP is different from PCP and PFC, +: PPP is different from PFC, †: PPP is different from PCP and PFC

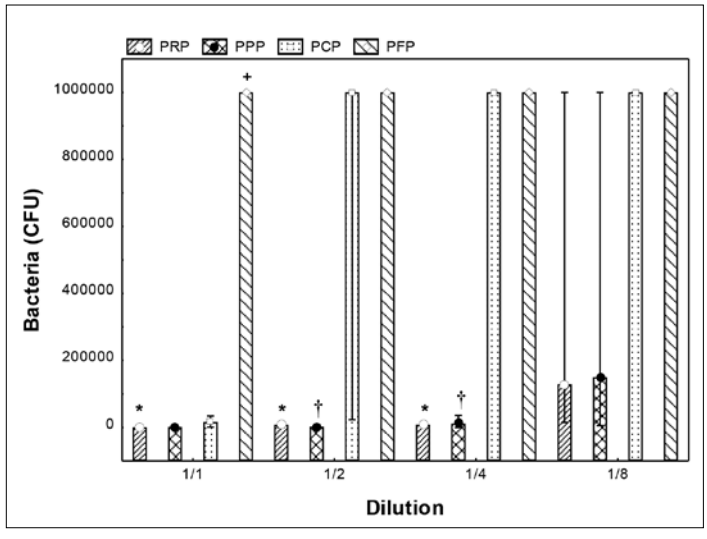

Figure 2. Comparison of the antibacterial effect of M. catarrhalis between all solutions and dilutions
*: PRP is different from PCP and PFC, +: PPP is different from PFC, †: PPP is different from PCP and PFC

Table 1. Comparison of antibacterial effect of *S. aureus* between all solutions and dilutions

	PRP	PPP	PCP	PFC	p	Post-hoc test p value
1/1	700 (0-925)	2100 (750-5975)*	9050 (1575-16500)	1000000 (100000-1000000)	0.001	PRP-PCP 0.044 PRP-PFC <0.001 PPP-PFC 0.014
1/2	200 (0-1725)	1500 (0-8250)	1000000 (794000-1000000)	1000000 (1000000-1000000)	0.001	PRP-PCP 0.004 PRP-PFC 0.002 PPP-PCP 0.025 PPP-PFC 0.014
1/4	0 (0-2600)	1400 (0-6500)	1000000 (1000000-1000000)	1000000 (1000000-1000000)	0.001	PRP-PCP 0.002 PRP-PFC 0.002 PPP-PCP 0.025 PPP-PFC 0.025
1/8	1600 (875-8500)	105000 (1275-1000000)	1000000 (1000000-1000000)	1000000 (1000000-1000000)	0.002	PRP-PCP 0.004 PRP-PFC 0.004

PRP: platelet rich plasma; PPP: platelet poor plasma; PCP: platelet-concentrated plasma; PFC: platelet-derived factor concentrate

Table 2. Comparison of antibacterial effect of *M. catarrhalis* between all solutions and dilutions

	PRP	PPP	PCP	PFC	p	Post-Hoc test p value
1/1	0 (0-250)	1350 (0-4450)	16150 (1900-29500)	1000000 (1000000-1000000)	0.001	PRP-PCP 0.034 PRP-PFC <0.001 PPP-PFC 0.003
1/2	8000 (900-10250)	2350 (0-8150)	1000000 (128000-1000000)	1000000 (1000000-1000000)	0.004	PRP-PCP 0.037 PRP-PFC 0.010 PPP-PCP 0.020 PPP-PFC 0.005
1/4	8450 (3900-24500)	11900 (1725-21750)	1000000 (1000000-1000000)	1000000 (768250-1000000)	0.001	PRP-PCP 0.003
						PRP-PFC 0.005
						PPP-PCP 0.010
						PPP-PFC 0.019
1/8	129000 (51050-1000000)	150000 (11125-1000000)	1000000 (1000000-1000000)	1000000 (1000000-1000000)	0.051	

PRP: platelet rich plasma; PPP: platelet poor plasma; PCP: platelet-concentrated plasma; PFC: platelet-derived factor concentrate

DISCUSSION

We investigated the antibacterial efficacies of PRP, PPP, PCP, and PFC obtained from whole blood on *M. catarrhalis* and *S. aureus*. Significant differences were found between PRP and PCP, PRP and PFC, PPP and PFC, and PCP and PFC in almost all dilutions; however, no significant differences were found between PRP and PPP and between PCP and PFC in nearly all dilutions. Also, concerning both bacteria, differences were determined in the antibacterial efficiencies of PRP, PPP, and PCP according to the dilutions.

When the efficacies against *S. aureus* and *M. Catarrhalis* were compared, no differences were found concerning PRP and PPP in the 1:1 and 1:2 dilutions. However, in the PRP 1:4 dilution, the efficacy against *M. Catarrhalis* was lower in a statistically significant manner.

The common characteristics of these platelet products are the involvement of platelets, leukocytes, and plasma. When PRP obtained as the first step is analyzed, it is determined that it contains a high amount of platelets and a minute amount of leukocytes.

In our study, PRP, PPP, PCP, and PFC, which were among the platelet plasma products prepared as recommended by Araki et al. (19), were used. While obtaining PRP, the complete sedimentation of platelets by centrifugation at low speed was not provided. However, while obtaining PCP by a second centrifugation at high speed, the total sedimentation of platelets was accomplished, and PPP was obtained by collecting the plasma; a PCP containing dense platelet concentrate was prepared subsequently. Following the centrifugation of PRP, the PPP portion of the obtained product was removed and replaced with phosphatebuffered saline in a 1/10 volume; the product was recentrifuged and activated by thrombin to obtain PFC. The primary purpose of removing the PPP portion completely was to eliminate the effect of fibrinogen. Platelet-derived growth factor BB (PDGF-BB) is a parameter nonexistent in the plasma and therefore is correlated with platelet activation. In the study conducted by Araki, this PDGF-BB was determined to have its highest value in PFC (19). In the same study, the total

Table 3. Comparison of antibacterial effect of all solutions on *M. catarrhalis* and *S. aureus*

		SA	MC	P
1/1	PRP	700 (0-925)	0 (0-250)	0.240
	PPP	2100 (750-5975)	1350 (0-4450)	0.485
1/2	PRP	200 (0-1725)	8000 (900-10250)	0.052
	PPP	1500 (0-8250)	2350 (0-8150)	0.818
1/4	PRP	0 (0-2600)	8450 (3900-24500)	0.009
	PPP	1400 (0-6500)	11900 (1725-21750)	0.093

PRP: platelet rich plasma; PPP: platelet poor plasma; PCP: platelet-concentrated plasma; PFC: platelet-derived factor concentrate

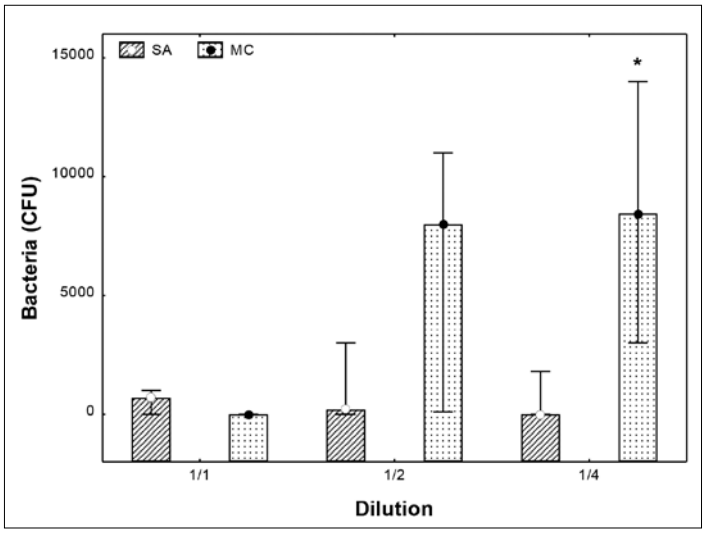

Figure 3. *In 1:4 dilution, the efficacy against Moraxella catarrhalis was found to be significantly lower for PRP

platelet ratios were found to be lower in PCP and PFC compared to PRP; this was suggested to be related to the partial loss and aggregation of the platelets at the second centrifugation. The PCP contains 7-fold more concentrated platelets compared to PRP. In our study, by determining the antibacterial efficacies of PRP and PPP and not observing this effect in PCP and PFC, we considered that this effect was not related to the platelet concentration or PDGFBB and was more likely to be linked to the other factors present in the plasma. In the study conducted by Tang et al. (20), seven antimicrobial peptides released from human platelets were identified. These were the platelet factor 4 (PF-4), RANTES, connective tissue-activating peptide 3 (CTAP-3), platelet basic protein, thymosin β-4 (Tβ-4), fibrinopeptide B (FP-B), and fibrino peptide A (FP-A) (20). These were shown to manifest an antibacterial efficacy against *S. aureus, Candida albicans, E.coli*, and *Cryptococcus neoformans* (20).

Platelet rich plasma (PRP) was determined to have a higher level of antibacterial efficacy against periodontal pathogens compared to the other plasma preparations (21). In another study, the antibacterial efficiencies of the platelet-rich and the platelet-poor PRPs on *S. aureus* and methicillin-resistant *S. aureus (*MRSA) were compared to whole blood and cefazolin; significant antibacterial efficacy was found in both when compared to whole blood, particularly in the 1h 4h, and 8h (22). The efficacies were reported to be reduced after 24 h. Therefore, it was suggested that they are effective in the first hours following surgery (22). However, they also reported that the efficacy against MRSA was reduced, and this may have been related to the penicillin-binding protein. No differences were found to be present at the twenty-fourth hour between the two PRPs and cefazolin concerning the efficacy against MRSA (22). While the effectiveness of the platelet-rich PRP was higher than the poor counterpart in the first hours, their effects were equalized in 24 h (22). Similarly, in our study, PRP and PPP had similar antibacterial efficiency against both *S. aureus* and *M. catarrhalis*. Irrespective of a low or high platelet count, the antibacterial effect against the two bacteria did not change. However, in a preparation where the platelets were more concentrated, such as PCP, the antibacterial efficacy decreased significantly. Therefore, the effectiveness of peptides present in the plasma was suggested to be at the forefront once more.

In another study, the antibacterial efficacy of PRP and PPP against *S. aureus* was determined at 1:8 dilution (2). In our study, we identified the antibacterial efficacy of PRP and PPP against both *S. aureus* and *M. catarrhalis* in all dilutions. However, different from our study, the antibacterial efficacies of both 2-fold and 4-fold concentrated PRP were determined against *S. aureus* at 1:16 dilution, but only platelets were found to be ineffective (2). Because the efficacy of platelets did not increase with their increasing concentration, it was emphasized that the antibacterial effect was more likely related to the plasma components in the same study. We determined that the antibacterial efficacy of PCP consisting of a platelet concentrate and small amount of plasma against *M. catarrhalis* was quite low. Moreover, the observed antibacterial effect with PFC in which PBS was used instead of plasma was a finding supporting the fact that the effect was related to the factors present in plasma.

The antibacterial efficacy has been reported to be reduced after the eighteenth hour (23). Therefore, because of its short-lasting effectiveness, recurrent administrations may be required in clinical use. In that study, PRP containing additional leucocytes was also used, and its antibacterial efficacy was found to be no different from PRP (23). We did not use preparations involving leucocytes in our study.

It was considered that the effectiveness against *M. catarrhalis* is less than that against *S. aureus* after dilutions of 1:4, which was another significant finding in our study, may have originated from the high resistance characteristics of this bacterium.

CONCLUSION

Although the present study was conducted in vitro owing to the determination of significant antibacterial effects of PRP and PPP against *S. aureus* and *M. catarrhalis*, it may be supportive of the suggestions that they can be used in addition to the conventional management, such as using as an intrasinusal lavage solution, when difficulties are encountered during the treatment of acute and chronic sinusitis. They also can be used for sinus lavage following endoscopic sinus surgeries. We consider that in addition to the antibacterial effect, using PRP and PPP in this manner may also be helpful in the regeneration of the inflamed areas. Further in vivo studies will be beneficial in this regard.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee for Non-Interventional Clinical Studies, Adnan Menderes University School of Medicine (25.12.2015, 2015/713).

Informed Consent: It is a culture study therefore informed consent is not taken.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - A.E., B.E, S.S.; Design - A.E., B.E, S.S.; Supervision - SS.; Resources - A.E., B.E., Z.B.; Materials - A.E., B.E., Z.B.; Data Collection and/or Processing- A.E., B.E., Z.B., S.S., İ.K.Ö.; Analysis and/or Interpretation - A.E., B.E., S.S., İ.K.Ö.; Literature Search - A.E., Y.B.; Writing Manuscript - A.E., Y.B., İ.K.Ö.; Critical Review - A.E., Y.B., B.E., İ.K.Ö., Z.B., S.S.

Conflict of Interest: The authors have no conflicts of interest to declare.

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