



## ORIGINAL ARTICLE

# In Vitro Evaluation of the Anti-bacterial Effect of Human Platelet Concentrate

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## ABSTRACT

**Background:** Recently the role of platelets in the tissue regeneration, wound healing and prevention and control of infections has been reported. We aimed to assess the antimicrobial effect of human platelet concentrate against six bacteria, commonly found in wound and hospital-acquired infections.

**Methods:** In vitro susceptibility to samples of 10 random human platelet concentrates was determined by disc diffusion method against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*. The assay was performed in triplicate for each strain and the antibacterial activities were assessed by measuring the zones of inhibition at 20, 24 and 48 hours after incubation at 37 °C.

**Results:** Human platelet concentrate showed antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* with the mean diameter zone of inhibition of  $11.4 \pm 1.1$  and  $10.2 \pm 1.1$  mm, respectively. Whereas, no activity was observed against *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus vulgaris*. Also, there was no significant difference in antibacterial effect of human platelet concentrate after 20, 24, and 48 hours.

**Conclusion:** Human platelet concentrate which is a biocompatible and safe product could be potentially useful in wound healing and hospital-acquired infections.

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## Introduction

Platelets are mainly known for their crucial role in the hemostasis.<sup>1</sup> Various recent studies have also indicated the important role of platelets in tissue regeneration, wound healing, and prevention and control of infection.<sup>2-7</sup> Therefore, nowadays platelet products are used in various fields of medicine, including dermatology, cosmetic and plastic surgery, ophthalmology, orthopedics, rheumatology, sports medicine and dentistry. Undoubtedly, such a widespread use of platelet products is largely related to the anti-inflammatory properties of platelets and the presence of multiple growth factors in these natural products along with their potential

antibacterial activity.<sup>1,8,9</sup>

Bacterial infections are among the most serious complications that provoke many social health concerns. Although the use of antibiotics is recommended for certain infectious situations, they can cause various adverse reactions. Improper usage of antibiotics may contribute to the increasing emergence of antibiotic resistance which has been referred to as one of the world's most pressing health problems.<sup>10</sup>

Considering the need for new effective, biocompatible and safe antimicrobial compounds, and since the antibacterial effect of platelet concentrate (PC) against some bacteria has been reported in a few in vitro

studies,<sup>2,8,11-14</sup> we aimed to investigate the antibacterial effect of PC against three gram negative and three gram-positive bacteria which are mainly responsible for wound and hospital-acquired infections.

## Methods

### Bacteria and Preparation of Inoculums

Three gram-negative bacteria including *Escherichia coli* (PTCC 1399), *Pseudomonas aeruginosa* (PTCC 1430), and *Proteus vulgaris* (PTCC 1079) and three gram-positive bacteria including *Staphylococcus epidermidis* (PTCC 1435), *Staphylococcus aureus* (PTCC 1431) and *Micrococcus luteus* (PTCC 1408) were selected for the study. The bacterial strains were obtained from Pasteur Institute (Tehran, Iran) and maintained on Nutrient agar at 4 °C at Islamic Azad University laboratory. To prepare inoculums of bacteria culture, the stock culture from Nutrient agar was subcultured on Muller-Hinton agar (Merck, Germany) and incubated overnight at 37 °C, then a suspension of freshly grown bacteria in sterile distilled water was prepared for each strain with an optical density equal to 0.5 McFarland ( $1 \times 10^8$  CFU/mL).

### Platelet Concentrate Preparation

Each of 10 random PCs was obtained from Tehran Blood Transfusion Center on the day of experiment. It should be noted that PCs prepared from whole blood of healthy blood donors using platelet-rich plasma method<sup>15</sup> were stored and shipped at 20 to 24°C along with continuous agitation during storage.

### Determination of Antibacterial Activity

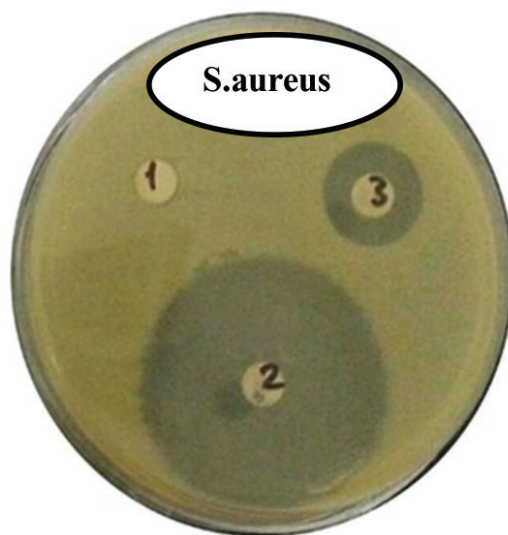
*In vitro* laboratory susceptibility to PC was determined by disc diffusion method<sup>16</sup> on Mueller–Hinton agar (MHA). For this purpose, agar plates were coated with one of the following bacterial strains: *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Micrococcus luteus* as Gram-positive bacteria and *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* as Gram-negative bacteria. Then standard 6 mm discs soaked with PC and positive or negative control were placed on the coated agar media. The inoculated agar plates were then incubated at 37 °C for 48 hours. The baseline antimicrobial activity was assessed by measuring the diameter zones of inhibition after 20, 24, and 48 hours after incubation at 37 °C and results were expressed as mean  $\pm$  SD. It should be noted that the assay was performed in triplicate for each strain and Penicillin and Gentamicin were used in all assays as positive controls for Gram-positive and Gram-negative bacteria, respectively. Mueller-Hinton Broth was used as a negative control.

## Results

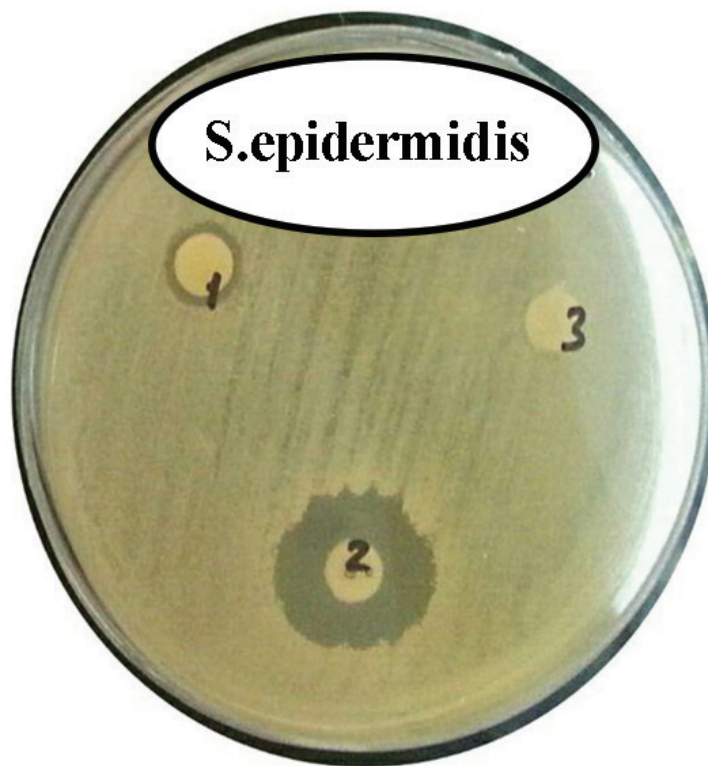
The mean values for zone of inhibition produced by PC, positive control and negative control against six bacteria are shown in table 1. PCs showed antibacterial activity against *Staphylococcus aureus* (figure 1) and *Staphylococcus epidermidis* (figure 2) with the mean diameter zone of inhibition of  $11.4 \pm 1.1$  and  $10.2 \pm 1.1$  mm, respectively. There was no activity against *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus vulgaris*. Moreover, there was no significant difference in antibacterial effect of PCs after 20, 24, and

**Table 1:** Zones of inhibition, exerted by platelet concentrate, positive control and negative control against six bacteria after 24 hours of incubation

Sample \ Bacteria	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus aureus</i>	<i>Micrococcus luteus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Proteus vulgaris</i>
Platelet Concentrate	10.2 $\pm$ 1.1 mm	11.4 $\pm$ 1.1 mm	-	-	-	-
Positive control	19 $\pm$ 2.0 mm	40.9 $\pm$ 1.8 mm	59 $\pm$ 3.7 mm	21 $\pm$ 2.1 mm	19.5 $\pm$ 0.7 mm	19.6 $\pm$ 0.7 mm
Negative control	-	-	-	-	-	-



**Figure 1:** The zone of inhibition exerted by 1-Mueller Hinton Broth (negative control), 2-Penicillin (positive control) and 3-Platelet concentrate against *Staphylococcus aureus* after 24 hours of incubation



**Figure 2:** The zone of inhibition exerted by 1- Platelet concentrate, 2-Penicillin (positive control) and 3-Muller Hinton Broth (negative control) against *Staphylococcus epidermidis* after 24 hours of incubation.

48 hours.

### Discussion

Despite a wide spectrum of available potent antimicrobials, bacterial infection remains a major problem. This is largely due to the emergence of bacterial resistance, caused by the inappropriate or inadequate use of antibiotics.<sup>10</sup> Therefore research for finding an alternative treatment and a solution for antibiotic resistance is crucial.

In the case of wound infections and hospital-acquired infections, it seems that platelet products could be appropriate adjuncts to antibiotics. Platelets can interact with microbial pathogens directly and indirectly through multiple molecular and cellular mechanisms. It has been suggested that platelets not only reduce incidence of bacterial infections but also promote wound healing.<sup>1,5,6</sup> Therefore, platelet products have recently attracted interest in this regard. But it seems that research in this field is still limited and insufficient. This study was designed to determine the in vitro antibacterial activity of human platelet concentrates against 6 common causes of wound and hospital-acquired bacterial infections.

The results of this study confirmed the previously reported antibacterial effects of human platelet concentrates against *S. aureus*.<sup>4,8,11,13,14,17</sup> The observed antibacterial activities of PCs against *S. epidermidis* were similar to findings of Anitua et al., while Burnouf et al. found different results in their research.<sup>3,8</sup> On the other hand, PCs were not effective against four other bacteria in our study. It should be noted that previous studies conducted on *P. aeruginosa* and *E. coli* yielded contradictory results.<sup>3,9,11</sup> PCs have not been tested against *M. luteus* and *P. vulgaris* yet.

It seems that donor's variability along with differences in the quality, viability, activation and degradation rate of platelets could cause variation in the susceptibility pattern of the gram-positive and gram-negative bacteria in comparison to other studies. Our study used PTCC bacterial strains which may behave in a way different from clinical isolates or ATCC bacterial strains. Therefore, further studies (both in-vitro and in-vivo) are needed to investigate the antimicrobial effect of platelet concentrates against viruses, fungi and other bacterial strains along with similar studies using clinical isolates.

### Conclusion

The findings of this study regarding the antibacterial effect of PCs against *S. aureus* and *S. epidermidis* were consistent with some other studies supporting the clinical use of platelets as a biocompatible and safe product in wound healing and hospital-acquired infections. Further research on PCs should be employed to determine exact antibacterial spectrum, their antimicrobial capacity along with antibiotics and their efficacy in in-vivo conditions.

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**Conflict of Interest:** None declared.

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