Abstract
Bacterial contamination of platelets concentrates (PCs) can result in transfusion transmissible infection. Storage temperature for platelets provides favourable environment for the bacterial growth. This study was conducted at Rawalpindi Institute of Cardiology, Rawalpindi, Pakistan from May, 2016 to July 2016. A total of 200 (48 hours stored) whole blood derived PCs collected were selected for the study. Sample were inoculated into Oxoid Signal blood culture bottles and incubated at 36±1°C for 07 days. Signal culture bottle with positive signals and visual appearance of turbidity were sub-cultured. Bacterial growth identification was carried out by standard reference methods. Out of 200 platelets concentrates, 63 suspected turbid and 02 with positive signal culture device were sub-cultured and identified. Staphylococcus aureus was identified in 02 bottles. The overall frequency of bacterial contamination in PCs was found to be 1%. The frequency of bacterial contamination in PCs found is very high as compared to developed counties. There is need of strict adherence to standard protocols for the prevention, early detection, and reporting of bacterial contamination in the PCs in Pakistan.

Keywords: Platelets concentrates; Bacterial Infection; Oxoid Signal blood culture.

Introduction
Transmission of transfusion transmissible viral infection like hepatitis C virus (HCV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV) have been reduced by the implementation of standard operating procedures for the donor enrollment and screening of Transfusion transmissible infections (TTIs) by highly sensitive screening methods like nucleic acid testing (NAT). However, Transfusion transmissible bacterial infection especially through Platelets Concentrates (PCs) is still one of the main sources of blood borne infections leading to high morbidity and mortality rate particularly in hospitalized patients.1,2

Bacterial sepsis due to transfusion of contaminated platelets is more common than due to transfusion of red cell concentrates. Approximately 18(16%) deaths have been recorded out of total 114(57%) TTIs reported due to transfusion of bacterial contaminated platelets.3 The bacterial contaminations of platelets units are approximately 1 in 1,000 to 3,000. However, the fatal sepsis is not clearly linked to the transfusion of contaminated platelets, but the probable mortality due to transfusion of bacterial contaminated platelets is 1 in 500,000.4

Microbes are present in nature and proficiently grow in the blood and its component. Platelets are more prone to obtain contamination due to their storage requirement.5 Storage temperature for platelets at 20-25°C with adequate nutrients provides favourable environment for the bacterial growth. It was reported in numerous studies that the PCs are mostly contaminated with bacteria.5

It is not generally simple to recognize the source of bacterial contamination in blood or its products. Donor’s skin bacterial flora like Staphylococcus aureus and Staphylococcus epidermidis might be one of the sources of bacterial contamination of platelets during the blood collection process.7

In Pakistan there is no study reported on bacterial contamination of platelets or other blood products despite the persistently increased demand of blood for children suffering with thalassaemia or haemophilia, in many surgical and obstetrical procedures and in road side emergencies. Complications due to transfusion of contaminated blood or its products although rare, but can lead to serious complications. Presently in Pakistan, screening of blood for bacterial contamination is not routinely carried out. Therefore, this study will not only create significant findings on the detecting bacterial contamination in platelets but also set a pathway to create standard operating procedures for detecting bacterial contamination in blood or its products.

Methods and Results
This is a single site cross sectional study which was carried
out at the Department of Pathology & Blood Bank of Rawalpindi Institute of Cardiology (RIC), Pakistan from May, 2016 to July 2016. RIC is a 272 bedded public sector cardiac hospital in Punjab province of Pakistan.

Total 200 whole blood derived platelets which were prepared by platelets rich plasma method were selected for this study. Sample size was calculated by using WHO and Raosft sample size calculator.8,9 The sample size was calculated by considering 5% probability of contamination of platelets concentrates at 95% confidence level. Ten ml samples were collected from 48 hours stored platelets using sterile syringes and aseptic techniques. The sample were inoculated into Oxoid’s Signal blood culture bottles and mixed thoroughly with the broth. The growth indicator device (for the detecting of positive pressure) was inserted in the bottle.

The signal culture bottles were incubated at 36±1°C for 07 days. During the incubation period, the culture bottles were inspected and mixed daily. Signal culture bottle with positive signals and visual appearance of turbidity were sub-cultured on Blood agar, Chocolate agar and MacConkey agar aerobically at 36±1°C for 48 hours. Bacterial growth identification was carried out by standard reference methods.10

Out of 200 platelets concentrates, 63 suspected turbid and 02 with positive signal culture device were sub-cultured and identified. Staphylococcus aureus was identified in 02 bottles (with positive signal) and the remaining 135 bottles were declared negative for bacterial growth on the basis of negative signal. The overall frequency of bacterial contamination in PCs was found to be (1%).

Discussion

Bacterial contamination of blood and its products is an exceptionally basic transfusion related hazard worldwide but unfortunately in Pakistan it was overlooked. Information regarding the prevalence rate of contamination in blood products and its source is very useful for arranging safety measures that reduce transfusion related sepsis or complications.

In our study the overall incidence of bacterial contamination in 48 hours stored PCs was found as 1%. The overall PCs contamination reported worldwide ranges from 0.01% to 12.5% (Table-1). Our reported results are on lower side as reported from low developed countries like Ethiopia 15(12.5%),11 Ghana 02(9.0%),12 Nigeria 14(8.8%),13 Zimbabwe 06(3.1%)14 and Tanzania 11(2.8%).15 However the prevalence rate is higher than developed countries like Belgium,16 Netherlands,16

**Table:** Frequency of platelets contamination in different countries.

<table>
<thead>
<tr>
<th>Name of Country</th>
<th>Frequency of Contaminated Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethiopia11</td>
<td>12.5 %</td>
</tr>
<tr>
<td>Ghana12</td>
<td>9.0 %</td>
</tr>
<tr>
<td>Nigeria13</td>
<td>8.8 %</td>
</tr>
<tr>
<td>Zimbabwe14</td>
<td>3.1 %</td>
</tr>
<tr>
<td>Tanzania15</td>
<td>2.8 %</td>
</tr>
<tr>
<td>Pakistan</td>
<td>1.0 %</td>
</tr>
<tr>
<td>Belgium16</td>
<td>0.74 %</td>
</tr>
<tr>
<td>Netherlands16</td>
<td>0.67 %</td>
</tr>
<tr>
<td>Australia17</td>
<td>0.18 %</td>
</tr>
<tr>
<td>China19</td>
<td>0.06 %</td>
</tr>
<tr>
<td>Germany18</td>
<td>0.05 %</td>
</tr>
<tr>
<td>New Zealand19</td>
<td>0.04 %</td>
</tr>
<tr>
<td>Norway20</td>
<td>0.03 %</td>
</tr>
<tr>
<td>USA21,23</td>
<td>0.02 %</td>
</tr>
<tr>
<td>Canada22</td>
<td>0.01 %</td>
</tr>
</tbody>
</table>

Australia,17 China,19 Germany,18 New Zealand,19 Norway,20 USA21,23 and Canada22 with the prevalence rate 797 (0.74%), 259 (0.67%), 544(0.18%), 05(0.06%), 218(0.05%), 24(0.04%), 11(0.03%), 156(0.02%) and 49(0.01%) respectively.

The Bacteria which are mostly linked with contamination of blood and its products include coagulase-negative Staphylococcus, Bacillus spp., S. aureus, Yersinia enterocolitica and Pseudomonas aeruginosa.24 The implicated organism in our study was Staphylococcus aureus but we did not identify the source of contamination. Poorly disinfected donor’s puncturing site, contaminated phlebotomy equipments, donor bacteraemia and tooth brushing prior to donation might be the source of contamination in blood products.25

The implicated organism is similar as reported in other literatures, the organism isolated in a study carried out in Ghana were coagulase negative Staphylococcus, S. aureus, and Bacillus spp., Yersinia enterocolitica, Citrobacterfreundii, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae.11 Makuni et al., in 2014, isolated S. aureus, coagulase-negative Staphylococci, E.coli and Bacillus sp. in thirty nine platelets samples.14 It is reported in many studies that good skin disinfection techniques, removal of first blood volume, early testing of bacteria, pathogen inactivation protocols and reducing the storage time of PCs can minimize the contamination of PCs.25

Conclusion

The findings of our study confirm that the bacterial contamination in Pakistan is high as compared to
developed countries. In order to improve blood safety in Pakistan, the standard protocols especially for the disinfection of donor’s skin and early detection of contamination in blood products should be followed. Furthermore, the awareness in the blood bank community about the source of contamination is a key to improve blood safety.

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**Reference**


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