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Research Article

BACTERIAL CONTAMINATION OF BLOOD PRODUCTS IN DIFFERENT REGION OF IRAN

Mohammadsadegh Latifi[1], *Khosro Issazadeh[1], Mohammad Reza Majid Khoshkholgh Pahlaviani[2]
[1]Department of Microbiology, Lahijan Branch, Islamic Azad University, Lahijan, Iran
[2]Department of Biotechnology, Lahijan Branch, Islamic Azad University, Lahijan, Iran
**Author for Correspondence*

ABSTRACT
Bacterial contamination of transfusion products, especially platelets, is a longstanding problem that has been partially controlled through modern phlebotomy practices, refrigeration of red cells, freezing of plasma and improved materials for transfusion product collection and storage. Bacterial contamination of platelet products has been acknowledged as the most frequent infectious risk from transfusion occurring in approximately 1 of 2,000–3,000 whole-blood derived, random donor platelets, and apheresis-derived, single donor platelets. Estimates of severe morbidity and mortality range from 100 to 150 transfused individuals each year. During a four-month period from January to mid-July of 2015, 92 samples (whole blood, platelet, plasma and Cryo) were collected. Each sample cultured in BHI medium was incubated in aerobic conditions for 7 days and was incubated for 1 to 2 weeks for anaerobic bacterial growth in anaerobic jar containing CO2, and were examined daily for bacterial growth. In respect of frequency of above mentioned isolated from whole blood and its products samples during total storage duration, greatest number was related to *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Escherichia coli* (9 cases). In the second step, greatest number was related to *Citrobacter* (7 cases) and in the third step, Coagulase-negative *Staphylococcus aureus*, *Streptococcus viridans* and *Klebsiella* has greatest number (6 cases).

Keywords: *Blood, Contamination, Plasma, Platelet*

INTRODUCTION
Now, we are at the beginning of the second century of utilizing human blood for therapeutic purposes. During 2 last decades, the fact that «the blood is a present that in the first glance is one of major developments of novel medicine» has been exposed to great pattern change taken the identification of novel pathogens. Transferable blood is a potential source of infection with known or unknown transferring agents. Since 1980, with recognition of infection to human immune deficiency virus (HIV), in order to mitigate people's fright, only the blood with zero contamination risk to transfuse to the people is acceptable politically. Given the decrease of viral transmission through allogenic blood. It can be stated that bacterial contamination remains as greatest threat. With concerns provided due to detection of infection transmitted by blood transfusion, this pattern changing led to revision of the quality of blood storing for transfusion. Due to the infections transmitted by blood, in the previous decade we were witnessing a dedicate revolution of therapeutic blood transfusion. Viruses, bacteria and other transferable organisms were frequently hazardous for blood recipients. During the studies conducted in various countries, the risks of blood bacterial contamination and spoiling have decreased through precise transportation and storage. Among the bacteria confronting in of contaminated blood transmission are gram negative bacteria including *Klebsiella pneumoniae*, *Escherichia coli*, *Yersinia enterocolitica*, *Pseudomonas fluorescence*, *Pseudomonas aeruginosa* and gram positive bacteria including Bacillus and Staphylococcus. Greatest contamination in red blood cell units is mostly created by *Yersinia enterocolitica*. Among other organism associated to red blood cells contamination are *Serratia marcescens*, *Serratia liquefaciens*, *Pseudomonas aeruginosa* and various species of Enterobacter. Results obtained from outbreak of blood cell products contamination with gram negative species are more catastrophic. Development of laboratory techniques for screening the blood products decreased the

bacterial factors. But, nowadays contamination of blood cell products with bacterial factors is considered as a major hazard in blood transmission medicine (Bano, 2010; Kang, 2005; Sen, 2000). In present study, we are addressing the bacterial contamination and antibiotic resistance of blood samples in blood transmission centers of eastern Gilan.

MATERIALS AND METHODS

During a four-month period from January to mid-July of 2015, 92 samples (whole blood, platelet, plasma and Cryo) were collected randomly from mobile blood transmission centers in Gilan province. So that, among these samples, 35 whole blood samples, 20 platelet reservoir samples, 22 plasma samples and 15 Cryo samples were evaluated in respect of bacterial contamination.

Methodology and Study Area

Present study was performed cross-sectionally in a number of mobile blood centers in Gilan province, and whole blood samples and its products (plasma, platelet and Cryo) were collected randomly from 3 blood transmission centers of Iran. Only the samples collected, examined and stored (up to one month) by blood transmission organization were used for blood transmission in these mobile centers. Expired samples and the samples probably positive for HIV, HBV, HCV and Syphilis were excluded.

Blood Samples Collection

All 92 blood samples collected aseptically following the standard bacteriologic techniques. 70% ethanol was used to disinfect the place of blood sampling on the tested blood bags. The blood stored in the blood bags was completely mixed and the end of bags was fastened. Then they were cleaned with disinfected using sterile swabs. After cutting the place with a sterile tweezers and discarding the clots (if present) along the cut sample, a little amount of blood of major blood bag was directed toward the available tube to enter the cut path. The end of each tube (line) was cut, then was isolated using Spencer forceps for blood returning and air flow to the major blood bag. For each blood bag, three knots were placed in blood taking place, so that after swabbing with 70% ethanol 1mL blood (stored whole blood, blood products including platelet, plasma and Cryo) was taken from third knot and were inoculated separately to 9 mL sterile (Brain Heart Infusion) culture medium.

Culturing and Detecting the Bacterial Isolates

Each sample cultured in BHI medium was incubated in aerobic conditions for 7 days and was incubated for 1 to 2 weeks for anaerobic bacterial growth in anaerobic jar containing CO2, and were examined daily for bacterial growth (in respect of sedimentation, hemolysis and turbidity). In day 2 and 7, one full sterile loop was taken from culture medium and incubated in blood agar, chocolate agar and MacConkey agar medium in aerobic and anaerobic conditions for a week in 37°. If colonies were present, their morphologies were examined and then bacterial identification was performed using gram staining technique and performing conventional biochemical tests such as carbohydrate fermentation tests and also in most cases using commercial kit Mini API 20E (Biomerieux Co., Marcei, France).

RESULTS AND DISCUSSION

Results

In respect of relationship between time periods of whole blood samples storage with bacterial contamination rate, it was indicated that greatest contamination (30%) was observed during 3 days period followed by one week period (25%) and finally more than one week period (19%). In respect of the relationship between blood product storage period and bacterial contamination percentage in all the sampled areas for lower than 3 days period, maximum percentage was related to the sampled of plasma, platelet and Cryo (21%, 18%, 14%), respectively. But for 3 to 7 days storage period, maximum contamination was related to platelet samples (28%), followed by plasma and Cryo samples (7%-18%). But for more than one week storage period, bacterial contamination rate was significantly related to stored platelet samples (31%), followed by plasma (9%) and Cryo (13%). In respect of the relationship between the total number of isolated microorganism with storage duration of whole blood and its products in all the sampling areas it was indicated that for whole blood sample, 19 positive sample were isolated while

storage, 11 positive sample were isolated during 3 to 7 days period and 5 positive samples were isolated in more than 7 days storage period (35 cases). But the positive cases isolated from platelet sample for 3 studied periods were 10, 5 and 5 cases, respectively. For plasma storage, these amounts were 9, 7 and 6 cases, respectively, and for Cryo sample, 6 cases were isolated during storage period, 5 cases for one week duration and 4 cases for more than one week storage period (Table 1).

Table 1: Microorganisms isolated and storage duration of whole blood, platelet and Cryo for all the sampling areas

Microorganism				**Storage Duration**
Total blood	**Platelet n=10**	**Plasma n=9**	**Cryo n=6**	Lower than 3 days
S. viridans	Coagulase- negative *Staphylococcus*(CNS)	*Staphylococcus aureus*	CNS	
S. pneumoniae	*S.viridans*	*S. viridans*	*S.viridans*	
S. aureus	*S.viridans*	CNS	-	
Whole blood (n=11)	**Platelet (n=5)**	**Plasma (n=7)**	**Cryo (n=5)**	
S.aureus	CNS	*S.aureus*	*S.viridans*	3 to 7 days
S.viridans	*Escherichia coli*	*S.viridans*	*S.viridans*	
S. pneumoniae	*Klebsiella*	*K.pneumoniae*	*Klebsiella* sp.	
CNS	*Citrobacter* sp.	*Klebsiella* sp.	*E. coli*	
E.coli	*S. aureus*	*E.coli*	CNS	More than 7 days
Klebsiella sp.	CNS	*Klebsiella* sp.	*S.viridans*	
Citrobacter sp.	CNS	*S.aureus*	*Escherichia coli*	
S.viridans	*Klebsiella* sp.	*S.pneumoniae*	*Klebsiella* sp.	

Table 2: Frequency of clinical isolates from whole blood samples and its products during storage period

Frequency	**Whole blood**	**Platelet**	**Plasma**	**Number**	**Cryo**	**%**
Microbial isolate						
S.aureus	2	2	3	2	9	9%
CNS	2	1	2	1	6	7%
S. pneumoniae	3	2	2	2	9	9%
S.viridans	1	2	1	1	6	7%
Escherichia coli	2	3	2	2	9	9%
Klebsiella	1	1	2	2	2	7%
Citobacter sp.	2	2	2	1	1	8%
Total	13	13	14	11	11	56%

In respect of frequency of above mentioned isolated from whole blood and its products samples during total storage duration, greatest number was related to *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Escherichia coli* (9 cases). In the second step, greatest number was related to Citrobacter (7 cases) and in the third step, Coagulase-negative *Staphylococcus aureus*, *Streptococcus viridans* and *Klebsiella* has greatest number (6 cases).

Discussion

Importance of outbreak and bacterial source contamination the blood and its product can't be overstressed in planning the preventive measures in the blood transmission centers across the world. Transmission of blood and its products in developed countries has a little danger for the patients, but it remains as a threat for probability of contamination (EL-Mahallawy *et al.*, 2005; FDA Guidance, 2001). In addition,

recognition of bacterial isolates, type of blood or contaminated components and antibiotic sensitivity pattern are of great importance of public health and affects on the clinical performance. Thus, given the results obtained from previous chapter, we will interpret and verify them in this chapter. In this regard, Adjei & opaco conducted a study and stated that probability and bacterial contamination of blood and its product has attracted little attention in Africa.

A few countries in Africa, such as Ghana (Bano, 2010; Sen 2000) and perhaps Kenya published some evidences on bacterial contaminations of blood and its products (Hiller, 2009). In present study, we will present a report on an 8.8% outbreak. This figure is lower than what occurred in China in western Africa. But it is comparable to 8.8% rate found in blood transmission of all the children of Kenya (Hiller, 2009). Very large value observed in this study like other reports on Africa, which indicate the urgent need for clinical and laboratory care for blood transmission in adults and children, clarifies the extent and nature of the problem. This will also indicates the need to improved measures to assure the safety of blood transmission. These attempts must performed in addition to advanced selection of donors and suitable screening and cleaning the blood taking area for humanist donors against the substitution or donors who mostly are present in these centers. Centers for disease control (CDC) also discovered two other cases with the same electrophoresis pattern. While CDC report indicates the lack of environmental contamination probability false positive laboratory results and skin contamination, but since skin disinfection was performed only by 70% alcohol, we can't include these factors completely form this report.

There is a need to improve the disinfection of donors' skin to meet recent who measures (Yazer and Triulzi, 2005). As previously was reported from Adjei *et al.,* (Bano 2010), we found that contamination is obvious during a week after blood donation. Gram positive skin bacteria can be isolated rapidly after blood donation. But this case is rare in stored bloods. While gram negative bacteria which are capable to tolerate the cold are usually unrecognizable up to a period after reproduction during the storing.

Presence of high resistance in these isolated organisms indicated growing significance of antimicrobial resistance worldwide (Schifman, 1993).

This demonstrates the need to develop disinfectant performance before blood transmission and antimicrobial treatment. In another study, bacterial contamination of blood or blood product with 3.5 % rate was observed which it is greatly different from near 30 % rates of present study, while the number of samples in two studies must be considered. In this study this rate is lower compared to other studies conducted in Africa such as7 % in Kenya (Hiller, 2009). 8.8 % in Nigeria (Datta *et al.*, 2012) and between 9 to 17.5 % in Ghana (Bano, 2010). This fact is justifiable given the arrangements of the countries and the exact time of regulations enforcement related to blood products. In any way, blood contamination in this study is higher than the value reported in developed countries. While contamination outbreak in USA is reported as 0.2 %, this value is 0.15 % in England (Ness *et al.*, 2001) and 0.1 % in France (Serious Hazard of Transfusion.

SHOT report for 2000). However blood contamination in developed countries is low, but serve annual out break of disease and mortality due to blood bacterial contamination is considered as a major factor of mortality and disease (Hoppe, 1992; Jacksonet *et al.*, 2003).

No study was performed so far in Uganda to determine the disease and mortality rate due to contamination blood transmission to the patients. *Staphylococcus aureus* and *streptococcus viridans* were organisms isolated in this study. Gram positive bacteria (*Staphylococcus aureus*, Coagulase-negative staphylococcus (CNS) and Bacillus sp.) and gram negative bacteria (*E.coli*, *Citrobacter freundii*, *Y.entrocolitica*, *Klebsiella pneumoniae*, *P.aeruginosa*) are isolates reported in the study conducted by Adjei in Ghana (Bano, 2010).

Opoku- okrah in Ghana also isolated only gram positive bacteria which are including Coagulase- negative staphylococcus, *Staphylococcus aureus* and Corynebacterium. *E.coli*, *Klebsiella pneumonia* is among the recognized gram negative bacteria (Sen, 2000).

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