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BLOOD BAG;
PROTECTION FROM BACTERIA: REVIEWt

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ABSTRACT… Transfusion of blood products is one of the key aspects of hospital care. Among the risk of blood transfusions, bacterial contamination of the blood bags is not so uncommon. Sources of bacteria can be several. Majority of the times the source of bacteria is the arm of the donor. Second important cause of bacterial contamination of blood transfusion, is bacteria in the blood stream of the donor. Donor is usually selected with strict selection criteria. Detailed history is usually taken. Diversion is another method used to reduce the bacterial contamination of blood bags. Temperature is also important parameter for the growth of bacteria. RBCs are stored at 4 ˚C and platelets are stored at 22˚C. For the ideal bacterial detection techniques, it is important that they can detect the bacteria as early and as low as possible. At the time of collection of sample for viral screening, number of bacteria might be too low to be detected.

Key words: Transfusion, BACTEC, Platelets, Venipuncture Bact/Alert.

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BLOOD BAG'S PROTECTION FROM BACTERIA: REVIEW

Transfusion of blood products is one of the key aspects of hospital care. Therefore, lot of emphasis has been laid to make the blood products safe for the recipient. Among the risk of blood transfusions, bacterial contamination of the blood bags is not so uncommon. First case of bacterial contamination was reported in 1941.[1] Then, the focus of the scientists was mainly on the prevention against viral transmission through transfusions. This can be well understood by the fact that the occurrence of bacterial contamination almost equals or even more than the contamination of blood products by human immunodeficiency virus (HIV), hepatitis C virus, hepatitis B virus and humanT-cell lymphotropic virus (HTLV) Several strategies has been planned to cope with the situation.[2]

In United States, deaths due to bacterial contamination of blood bags, was the one of the leading cause in 1990s. Situation was the same in France in 1990s. In UK also, bacterial contamination was extensively reported between 1995 and 2003.[3]

SOURCE OF BACTERIA

Sources of bacteria can be several. Majority of the times the source of bacteria is the arm of the donor. Disinfection of the donor's arm is done but it does not eliminate bacteria from the arm. This is because of the fact that superficial layer of skin is disinfected with this technique but the deeper layers are not disinfected. Therefore, when needle is inserted into the skin, it takes bacteria from deeper skin into the needle, which enters the blood bag with blood. This process is facilitated at the dimpled sites.[3]

Second important cause of bacterial contamination of blood transfusion, is bacteria in the blood stream of the donor. Usually, such donors are excluded due to fever. However, asymptomatic patients can be the actual cause of such bacterial contamination. Long term infections can also be the reason of such bacterial contamination. Good example of this is the Yersinia enterocolitica. This bacterium lives in the intestine. It can enter the body through oro-fecal route. It multiplies best at 4˚C (Temperature at which RBCs are stored). 50 % of cases of bacteremia of donor with this bacterium are asymptomatic. Campylobacter

jejuni, Salmonella Heidelberg, Salmonella enterica and Escherichia coli are also important for bacteremia.[3,4]

Salmonella enterica and Serratia liquiefaciens are known to cause low grade chronic infection. Staphylococcus aureus is one of the most common bacteria that lead to transient bacteremia. Tooth extraction and electronic toothbrush usage is one the frequent causes of transient bacteremia. Both low grade infections and transient bacteremia leads to bacteremia and ultimately bacterial contamination of transfusion bags.[2] Serratia marcescens was also once isolated as a source of contamination of transfusion. This bacterium was present on the transfusion bags and it can multiply easily at 22 degree Celsius and 4 degree Celsius.[2] Blood processing can also be a danger for transmission of bacteria in blood bags. Damage can occur to blood bags during processing. Lack of sterility of welding machines of tubing can be a risk for bacterial transmission in bags. Similarly, contaminated water in water baths can also be a source of bacterial contamination.[3]

In comparison, pooled platelets (which are collected from several donors) are more prone to carry bacteria than platelets achieved through apheresis. This is due to the fact that more venipuncture are done in case of pooled platelets, so more chances of getting infected with bacteria of skin. RBC bags are contaminated lesser than platelet bags. This is because that Red Blood Cells (RBCs) are mainly stored at 4˚C while platelets are stored at 22 4˚C. In platelet bags, the bacterial number, which can cause reaction, is not well established. Nevertheless, in few studies, it has been shown that bacterial number can rise to 10^6 to 10^{11} cfu/ml (McDonald and Blajchman, 2008). Different bacteria isolated from platelet and RBC bags are shown in Table-I and II.

METHODS TO PROTECT BLOOD PRODUCTS

Donor is usually selected with strict selection criteria. Detailed history is usually taken. To prevent the transmission of Y. enterocolitica, donors with history of gastroenteritis illness are usually excluded. Puncture site is carefully selected to avoid any infected site. Almost half of donors have 10^5 organisms/cm^2 at the site of venipuncture. Then, disinfection of skin is done with utmost care. First few milliliter of blood is diverted as a precaution. Coagulase-negative Staphylococci, Staphylococcusaureus, Corynebacterium sp., Propionibacterium acnesandBacillus sp., top the list of bacteria that are transmitted through skin in the transfusions.[5,6]

Organism	Total number of Transmissions	Deaths reported
Staphylococcus epidermidis	20	1
Staphylococcus aureus	7	1
Bacillus cereus	7	3
Coagulase negative Staphylococcus	4	0
Streptococcus species	4	0
Group B Streptococcus	3	1
Propionibacterium acnes	3	0
Enterococcus faecalis	1	0
Total Gram-positive organisms	49 (68%)	6 (37%)
Escherchia coli	9	3
Serratia species	4	3
Enterobacter species	4	1
Klebsiella species	2	1
Yersinia enterocolitica	1	0
Morganella morganii	1	1
Acinetobacter species	1	0
Proteus species	1	1
Total Gram-negative organisms	23 (32%)	10 (63%)
Total	72	16

Table-I. Summary of Bacteria in Platelet Bags [3]

Organism	Total number of Transmissions	Deaths reported
Coagulase negative Staphylococcus	4	0
Staphylococcus aureus	2	0
Bacillus cereus	2	0
Staphylococcus epidermidis	2	1
Streptococcus species	2	0
Enterococcus faecalis	1	0
Propionibacterium acnes	1	0
Total Gram-positive organisms	14 (47%)	1 (14%)
Acinetobacter species	4	1
Serratia species	4	2
Yersinia enterocolitica	2	1
Pseudomonas species	2	1
Escherchia coli	1	0
Enterobacter species	1	1
Klebsiella species	1	0
Proteus species	1	0
Total Gram-negative organisms	16 (53%)	6 (86%)
Total	30	7

Table-II. Summary of Bacteria IN RBC Bags [3]

This can be well imagined by looking at the data that 90 % of bacterial transmission through platelets and 70% of bacterial transmission through the RBCs is due to bacteria present on the skin. Therefore, several procedures are being adopted to avoid this skin contamination before blood transfusion. Among them first procedure is decontamination by 70% isopropyl alcohol followed by tincture of iodine. Other one is to apply 70% isopropyl alcohol for 30 seconds and then by 2 % chlorhexidine gluconate. Some centers use only chlorhexidine alcohol. Apart from type of disinfectant used, other factors also play their role in deciding the control of skin infection. Among them, concentration, quantity and time of substance applied for disinfection, are important. Patient and phlebotomy people should be aware of the fact that skin disinfection is important.[3]

Diversion is another method used to reduce the bacterial contamination of blood bags. In this method, initial few milliliter of blood is diverted. This blood is also utilized for tests like serology. This process with skin disinfection leads to reduction of bacteria to 77%. However, it should be noted that bacteria cannot be eliminated by this procedure.[7]

Temperature is also important parameter for the growth of bacteria. RBCs are stored at 4 ˚C and platelets are stored at 22˚C. There is discussion to reduce the temperature of the blood bags. Therefore, RBC temperature is planned to reduce to 0 ˚C, although, 4 ˚C is less favorable for growth of bacteria. Platelets are stored at 22 ˚C, which is favorable for growth of bacteria. Researchers are trying to reduce platelet's storage temperature to 4 ˚C. However, the post transfusion count is not good after this low temperature storage.[8] Storage time of blood bags are also important deciding factor for the growth of bacteria. It has been observed that more transfusion reactions have been reported with storage time of more than 5 days for platelet bags. Similarly, storage time has been suggested to be reduced to 25 days to control growth of Y. enterocolitica. Nonetheless, reducing the time has a drawback that it limits the supply of blood. Leucodepletion filters have been used in many centers. It reduces the number of bacteria or it can cause damage to bacteria.[7]

Overnight stay has been implicated in few centers. It has been shown that overnight stay of 24 hours before component preparation can reduce the bacterial growth by antibacterial activity of cytokines. On the other hand, the quality of components is not so much affected. Still, further studies are needed to finalize its effects.[7] In spite of all these efforts, actual solution lies in reduction of transfusion. It can be achieved by increasing the trigger for transfusion and doing regular audit of transfusion. Similarly, apheresis transfusion

should be prioritized.[3]

IMPORTANCE OF DETECTION OF BACTERIA

In comparison to viral detection methods and success, bacterial detection is still in the beginning. Bacterial detection is not same as that of viral screening in several aspects. Bacteria can multiply in blood bags while viruses cannot. The difference between the growth of bacteria and viruses is that the viruses need the host cell to divide for its division. This is the reason that viruses has less chance to multiply in packed red cells, platelet bags and fresh frozen plasma. Nevertheless, bacteria can grow very easily and rapidly in the platelet bags at 22 ˚C.[7]

Secondly, bacterial load can be too low to be detected, when the blood is collected. Other difference is the diversity of the types of bacteria, which can infect the blood bags. Bacterial species are usually environmental which precludes the antibodies and hence their detection through antibody detection.[7]

The tests, which are available, are not rapid, sensitive, simple and specific. Ideally, these tests should be done early for the detection of the bacteria in contrast to viruses. As the bacteria can grow so the tests should be done regularly at intervals to ensure the negativity, which is not the case in viruses. In addition, all the components should be checked for bacterial growth. While in viral screening one time, testing is sufficient for whole blood. Donor exclusion has the limited advantage in case of bacterial contamination of blood bags.[7]

Bacterial growth has a peculiar pattern. It starts from lag phase, in which bacteria are not multiplying. In this phase, detection of bacteria can be an uphill task if the number of bacteria is low. Usually the number of bacteria is low at the time of collection of blood. Then the bacteria enter the other phase of multiplying. Single bacterial colony can give rise to millions of bacteria in few hours. This exponential phase ends due to lack of nutrients or accumulation of toxins. Therefore, the bacteria entering the blood bag multiplies or die depending on the environment of blood bags. Platelet bags are more suitable for growth of bacteria.[7]

For the ideal bacterial detection techniques, it is important that they can detect the bacteria as early and as low as possible. At the time of collection of sample for viral screening, number of bacteria might be too low to be detected. If they are, allow multiplying in culture, and then it takes hours or days to do so. Until then the bacteria in blood bags may die. Other option is to take sample after 24 hours from blood bag, which will allow bacteria to multiply and then to be detected easily. Alternatively, culture can be done initially followed by rapid technique after few days.[3]

METHODS OF BACTERIAL DETECTION

Visual Inspection of Blood Bags

Colour changes occur in blood bag owing to the contamination of blood bags. It usually occurs at amount of bacteria of about $1.8x10^4$ to $1.6x10^9$ cfu/ml. This colour change occurs in RBC bag due to consumption of oxygen, which leads to desaturation and hemolysis. Platelets have particular shine due to discoid shape that gives swirling visual effect. So, in platelet bags swirling is seen to know about the contamination of bacteria. Because more the bacteria, lesser will be the pH and hence the swirling of the platelet bag. Nevertheless, in several studies it has been mentioned that swirling has very low specificity. Swirling can decrease even in the absence of bacteria.[7]

Microscopic Examination

Gram staining and Acridine Orange are mainly used to detect the bacteria. They require bacteria at the range of 10^6cfu/ml and 10^5cfu/ml for detection. These tests can be done before transfusion. However, they lack sensitivity and specificity.[7]

Molecular Techniques

Molecular techniques are deemed sensitive and specific among the researchers. However, broad

spectrum of bacteria is still considered one of the limitations for this technique. It detects mainly ribosomal ribonucleic acid (RNA) at range of 10^5–10^9 cfu/ml. Still commercially, it is not available for routine use.[7]

Endotoxin Detection

Gram-negative bacteria cell wall lipopolysaccharide can lead to endotoxin mediated septic shock syndrome. Limulus amoebocyte lysate (LAL) test uses LAL reagent formed from horse shoe crab haemolymph, is considered highly sensitive and specific in diagnosing the toxic reaction due to gram-negative bacteria. This test can detect bacteria in blood bags in the range of 10^1 to 10^5 cfu/ml. The limitation of this test is that it cannot detect the gram-positive bacteria in blood bags.[7]

Automated Blood Culture Systems

This automatic system for detection of bacteria was designed for microbiology laboratory. Sample is loaded in aerobic or anaerobic culture bottles and then incubated in the system. If bacteria will be present, it will generate carbon dioxide. This change in bottle is detected by the system and alarm is rung. BACTEC, BacT/ALERT and ECP are in use.[7]

RAPID TECHNIQUES

Scan system

Scan system is Food and Drug Administration (FDA) approved for the detection of bacteria in platelet bags. Sample for 3 platelet bags is collected and put in the system. It has fluorescent double stranded Deoxyribonucleic acid (DNA), which can bind to the bacteria. In addition, platelets ate aggregated by reagent. Then the material is filtered, which filters platelets and allows bacteria to pass. These bacteria are then observed under fluorescent microscopes, if present. Sensitivity of this test is observed to be almost 10^3 cfu/ml.[8]

Dielectrophoresis

In this system, platelets are placed in system. Platelets are attracted to the electrodes depending on the dielectric properties of the conductivity and permittivity. If bacteria are present, they can be detected by impedance of the current. This system can detect 10^4 cfu/ml to 10^5 cfu/ml bacteria, depending upon their strains.[8]

pH and Glucose

Bacteria can metabolize glucose, which results in release of acid. Dipsticks can rapidly and easily detect both of these changes. It can detect 10^5–10^8cfu/ml bacteria. Though, this method lacks specificity.[8]

Flow cytometry

Bacteria are stained with fluorescent dye thiazole orange. Stained bacteria are then detected by flow cytometry through argon laser. It is rapid technique with detection capability of 10^4cells/ml. Large load of bacteria should be there for detection.[8]

Bacterial Cell wall detection

The Pan Genera Detection (PGD) assay can find the lipoteichoic acid of Gram-positives and lipopolysaccharides of Gramnegative bacteria by the use of antibodies. Another method uses enzyme immunoassay reader. These methods are still in development and they can provide point of transfusion detection of bacteria.[8]

Pathogen Reduction Technologies

Pathogen reduction technologies are undergoing lot of research. These are used to prevent the growth of the bacteria by interfering with the DNA or RNA. This method is good in blood products due to lack of DNA in RBCs, platelets and Fresh Frozen Plasma (FFP). It also helps to reduce the leucocyte in blood bags.[8] Amotosalen-HCl (AH) and Riboflavin are mainly used for platelet transfusion bags. Whiel PEN-110 and S-303 are mainly used for RBC transfusion bags. Different aspects of these pathogen reduction technologies are being extensively studied.[8]

Platelets and Bacterial Growth

Platelet bags are more prone to develop bacterial contamination. It has been shown that the risk of bacterial infection is 50-250 times more than viral infection. Between 2005 and 2009, bacterial infection was considered third main cause of

fatalities due to transfusion. This high bacterial contamination of platelet bags is due to the porous platelet bags and storage at 22˚C. Both of these aspects are ideal for growth of bacteria.[9] Due to this fact platelet, storage time was reduced to 4 days in several centers. In the United States, platelet storage time was reduced to 5 days. While in the Japan, it was reduced to 3 days. Then with the implication of bacterial detection methods the storage time of platelets was increased to 7 days in few centers.[7,10] American Association of Blood Banks (AABB) implicated the detection of bacteria in platelet bags in 2004 in its standard 5.15.1 Bacterial detection is mandatory in Belgium and Netherlands since 1998 and 2011 respectively.[11]

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AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Dr. Hammad Tufail Chaudhary	Designing of the work, acquisition, analysis, interpretation of data and drafting the work.