



Original Research Article

Rapid Detection of Bacterial Contamination in Platelet Concentrates, by Polymerase Chain Reaction and DNA Sequencing in Comparison to Conventional Automated Culture

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ABSTRACT

Keywords

Platelet concentrate;
bacterial contamination;
automated culture;
PCR;
DNA sequencing.

Bacterial contamination is a major cause of morbidity and mortality in platelet concentrate (PCs) transfusion all over the world. To develop a PCR method for sterility testing of PCs that is sensitive, specific for all relevant bacterial species; also to determine the prevalence of bacterial contamination, types of organisms and their antibiotic susceptibility. A randomly selected 56 PCs on the fifth day of donation were included in the study; 20 apheresis single and 36 random donor units. Bacterial contamination was detected by BACTEC 9050 culture system and PCR of a conserved region of the bacterial 16S rDNA, followed by DNA sequencing. Out of the 56 platelet units, 7 were positive by both methods and 3 by PCR alone with highly significant association ($P < 0.001$). All positive units were random donor. Isolates were *Staphylococcus epidermidis* ($n=3$), *E. coli*, *Klebsiella*, *Staphylococcus aureus* and *Acinetobacter*. By PCR, additional two *E. coli* and one *Klebsiella* were detected. PCR had 100% sensitivity and 93.9% specificity. All *Staphylococci* and *Acinetobacter* were multi-drug resistant. PCR has an excellent sensitivity and shorter turnaround time for detection of bacterial contamination of PCs. Thus sterility check, platelet apheresis and proper infection control measures are mandatory.

Introduction

For blood transfusion, blood products are routinely screened by ultra-sensitive methods to reduce the risk of virus transmission. Bacterial contamination is still the major cause of transfusion-related infectious complication (Ribault *et al.*,

2004; Carr-Greer, 2012). Contamination of platelet components is considered the second most common cause of transfusion-related deaths in the United States (CDC, 2013).

Platelet concentrates (PCs) carries the greatest risk for bacterial contamination due to their aerobic storage at 20-24°C to maintain optimum viability and function of platelets. The estimates are 50 to 250 times higher than the combined risk of infection with human immunodeficiency virus, hepatitis B virus and Hepatitis C virus, and human T-cell leukemia virus type 1 and 2 after blood transfusion (Dreier *et al.*, 2004; Ribault *et al.*, 2004; Canellini *et al.*, 2010). Up to 1000 cases of clinical sepsis after platelet transfusion are reported every year in the United States. The actual risk of transfusion-associated sepsis is higher due to under-reporting and unrecognition of infected cases. The condition is characterized by acute reaction symptoms and the rapid onset of septicemia that carries a 20 to 40% mortality rate (Hillyer *et al.*, 2003; CDC, 2013). Platelets are often given to pancytopenic immunocompromised patients that do not have the ability to fight infection. Therefore, early detection of bacterial contamination in platelet concentrates is of great value for patients in need for repeated platelet transfusion (Leon *et al.*, 2008).

Since March 1, 2004, The American Association of Blood Banks (AABB) adopted effective standards to limit the potential for bacterial contamination during collection of platelet units and for detection of bacteria prior to their release or issue (Hillyer *et al.*, 2003; Carr-Greer, 2012). The sterility screening of PCs is mandatory in some countries as Belgium (since 1998), Netherland (since 2001) and USA (since 2004) (Dreier *et al.*, 2004). Although the methods to limit and detect bacterial contamination of PCs are implemented, this does not eliminate the risk of infection (AABB, 2013). The implementation of early platelet culture

has decreased the post-platelet transfusion-associated fatalities up to 60% to 83% (Brecher *et al.*, 2013).

Different methods for detection of bacterial contamination in PCs include automated culture (gold standard), bacterial morphology, biochemical, immunological and molecular methods. A proper test should be rapid, affordable, sensitive and simple to perform. Although automated culture is very sensitive and detects many types of microorganisms. Still, it requires prolonged periods of time (2 to 4 days). Molecular methods for detection of bacterial genes were developed with high sensitivity (Dreier *et al.*, 2004). The aim of this study was to develop a PCR method to detect the bacterial contamination in platelet concentrates that is sensitive, specific for all transfusion-relevant bacterial species, and more suitable for routine sterility testing of platelet concentrates (PCs); Also to determine the prevalence of the bacterial contamination of PCs, the types of the involved organisms and their antibiotic susceptibility pattern.

Materials and Methods

The present study was conducted during a two years period from October 2006 to October 2008 at the Main Blood Bank, Ain Shams University Hospitals, Cairo and the National Blood Transfusion Center in Cairo, which is a governmental non-profit organization under the full authority of the Ministry of Health and Population in Egypt. Bacterial contamination in platelet concentrates was detected by automated culture as a gold standard (Yomtovian *et al.*, 2006; Korsak, 2012) and by PCR and DNA sequencing. Automated culture was performed in the Microbiology Laboratory of Ain Shams

University Hospitals. All molecular techniques were done at The Molecular Epidemiology Department, U.S. Naval Medical Research Unit No.3 (NAMRU-3).

Specimen collection

The study involved 56 platelet units that were randomly selected on the fifth day of donation. Twenty apheresis-derived single donor platelets units and thirty six random donor platelets were conducted in our study. Blood donors were selected according to the AABB questionnaire (2005). All the units were sero-negative for HCV, HBV, HIV and syphilis.

Samples from the platelet units were collected on the fifth day of donation using 10 mL sterile syringe after alcohol swabbing. From each platelet sample, 5ml aliquot was cultured in automated blood culture system; 2ml aliquot was stored immediately at -70°C for PCR amplification of the 16S rRNA gene and DNA sequencing to detect the presence of microorganisms. A direct Gram-stained smear was also examined for detection of microorganisms by their staining character.

Processing

Platelet culture technique

Five milliliters of each platelet sample was cultured using the automated blood culture BACTEC 9050 system (Beckton Dickinson, USA) and incubated according to the manufacturer's protocol at 37°C for five days. Samples from the positive signal blood culture bottles were obtained after shaking under aseptic conditions. The sample was then centrifuged, and the deposit was used for Gram stain and sub-cultured on two blood agar plates (Oxoid, UK) (aerobic and anaerobic) as well as on

MacConkey agar plate (Oxoid, UK) and incubated at 35°C overnight. Growing organisms were subsequently identified by routine methods and API systems (bioMérieux, Marcy L'Etoile, France) and their antimicrobial sensitivity was determined by disc diffusion method (Koneman *et al.*, 2006). Negative bottles were removed after 5 days from the instrument and processed as previously mentioned before being discarded to confirm absence of microorganisms.

Antimicrobial disk diffusion test

All the isolates detected by automated culture were tested for antimicrobial susceptibility according to the recommendations of the Clinical Laboratory Standard Institute (CLSI) (2008). The antibiotic disks (Oxoid, UK) were used on Mueller Hinton agar (Oxoid, UK). The inoculum turbidity was adjusted to 0.5 McFarland. Then the agar plates were inverted and incubated at 35°C for 24h.

Amplification of the bacterial 16S rRNA gene by polymerase chain reaction (PCR)

DNA extraction

The genomic DNA was extracted using a DNA Purification, QIA amp DNA Mini Kit supplied by (Qiagen, USA) following the manufacturer's instructions. For cell lysis, 400µl of the platelet suspension was added to 1.5 mL microfuge tube with 40µL Proteinase K and 400µL AL buffer, and incubated at 56°C for 1h. Then 400µL Ethanol (96-100%) was added and vortexed. The Lysate was added to QIA amp Spin column and centrifuged at 8,000 rpm for 1min and this step was repeated. 500 µL of AW1 Buffer was added then

centrifuged at 8,000 rpm for 1min followed by 500 μ L of AW2 Buffer and centrifuged at 14,000 rpm for 3min. The QIA amp spin column was placed in a new 2mL collection tube and centrifuged at 14,000 rpm for 1min. 200 μ L of AE Buffer was added then incubated at room temperature for 1min and then centrifuged at 8,000 rpm for 1min.

DNA amplification

Five microliters of the DNA template was added to the PCR mix (Promega, USA) which contained 10 μ l 5x Taq buffer, 3 μ l 25mM MgCl₂, 4 μ l 2.5mM PCR nucleotide mix, 1 μ l 30 μ M 8–27 Forward primer, 1 μ l 30 μ M 806 Reverse primer, 0.5 μ l Taq DNA polymerase, and 25.5 μ l sterile nuclease free water. Sterile nuclease free water was used as negative control in each PCR run. Oligosynthetic DNA, prepared by Genesearch (Australia), was used as a positive control. The amplification was then performed by including the reaction mix for 35 cycles into a thermalcycler, Gene Amp PCR system 9700 (Applied Biosystems, USA). Each cycle consisted of denaturation at 95°C for 30 seconds, followed by annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds with an initial delay for 10 minutes at 95°C at the beginning of the first cycle and a 7 minutes delay at 72°C by the end of the last cycle followed by soak at 4°C (Jalva, 2000). The primers (Promega, USA) used for the amplification of the 16S rRNA gene are listed in Table (1).

Detection of the amplification product

Ten microliters of each of positive control, negative control, PCR amplicons and 5 μ l of the molecular size DNA ladder (Promega, USA), were mixed with 2 μ l of

6x gel loading dye (Promega, USA) and the mixture was electrophoresed on 2% agarose gel (Sigma, Germany) in Tris-acetate EDTA buffer (2M Tris-HCl, 0.05M EDTA, adjusted to pH 8.0 by glacial acetic acid) and stained with ethidiumbromide (Amersco, USA). The gels were viewed under ultraviolet light and photographs were taken.

Interpretation of the PCR results

The molecular size marker gave different bands ranging from 100bp-1600bp (Promega, USA). The negative control was examined to exclude any source of contamination. Samples that yielded amplified products corresponding to 400bp were considered positive for the 16S rRNA gene (Jalva, 2000) (Figure 1 & 2).

DNA Sequencing of the Amplified PCR Product

The PCR products were sequenced by the dideoxynucleotide chain termination method (Sanger *et al.*, 1997) in an automated DNA sequencer Genetic Analyzer 3100 (Applied Biosystems, USA). DNA sequencing and analysis included the following steps:

Purification of the PCR products

It is done by QIA quick PCR Purification Kit (Qiagen, USA) according to the manufacturing instructions. Two hundred microliters of PBI buffer was added to 40 μ L of the PCR product and centrifuged to bind DNA in a QIA quick spin column for 1 minute at 13,000 rpm. A 750 μ L of PE Buffer was added to the QIA quick column and centrifuged for 1min at 13,000 rpm. After discarding the flow-through, centrifugation for additional 1min was

done. The QIA quick column was placed in a clean Eppendorf. To elute DNA, 40 μ L of EB Buffer (10mM Tris-Cl, pH 8.5) was added and centrifuged for 1 min at 13,000 rpm.

Cycle sequencing reaction (fluorescent dye labeling of PCR products)

Principle: A simple method in which 25 successive cycles of denaturation, annealing and extension results in linear application of fluorescent dye labeled extension product by using labeled (ddNTPs) dye terminator. Taq polymerase extends the primers incorporated ddNTPs that stops the extension reaction to randomly generate fragments that differ in length by one base.

Procedure

Cycle sequencing reaction was done using the ABI Prism Big Dye Terminator V3.1 Cycle Sequencing Ready Reaction Kit (PE Applied Biosystem Com, Cal, USA). Four microliters of the PCR products, containing the amplified genes, were added to 16 μ l reaction mixture containing 2 μ l Terminator ready reaction mix (fluorescein-labeled dye terminators), 2 μ l Primer, and 8 μ l deionized water in a total volume of 20 μ l. The mixture was then placed in the Gene Amp 9700 Thermocycler (Applied Biosystems, USA) which was programmed for 25 cycles; each consisting of rapid thermal ramp to 96°C for 10 sec, 50°C for 5 sec then 60°C for 4 min (McMurray *et al.*, 1998).

Purification of labeled extension products from excess unincorporated dyes

Complete removal of the unincorporated dye terminators was done before sample

electrophoresis to avoid masking of data in the early part of the sequence as well as interference with the base calling. This is achieved by the dye Extraction2.0 Spin kit (Qiagen, USA) which is designed using gel filtration technology in a convenient microspin format (Eppendorf centrifuge 5417R, Germany) to allow cleaning of sequencing reaction in just 7 minutes. The spin column was vortexed gently and centrifuged for 3min at 7,000rpm. 20 μ l of the sequencing reaction mixture was applied onto the slanted gel-head surface and centrifuged for 3min at 7,000rpm. The mixture was heated at 85°C for 30min; 12 μ L Hi-Di formamide (Applied Biosystems, UK) was added, heated at 95°C for 5 min and finally transferred to ice till analyzed by capillary electrophoresis.

Loading of the purified labeled extension products on the ABI prism 310 Genetic Analyzer for determination of gene sequence

Ten microliters of the purified elute was loaded in the reaction well of the auto-sampler tray. The AB 3100 Genetic Analyzer then analyzes the fluorescently labeled DNA fragments by capillary electrophoresis and the base calling view of the DNA sequence was obtained for each case (Figure3) (Lee *et al.*, 1997).

Data reading and interpretation

Sequence editing was performed using the BioEdit Program V.5.0 and data obtained were compared to the sequence obtained from the GenBank data base (<http://www.ncbi.nih.gov/BLAST>).

Statistics

Categorical variables were expressed as

number (%). The sensitivity, specificity and the positive and negative predictive values were calculated. All the analyses were performed with commercially available software (SPSS version 16.0, SPSS, Inc., Chicago, IL, USA).

Results and Discussion

Fifty six platelet units were collected from the Main Blood Bank, Ain Shams University Hospitals and the National Blood Transfusion Center. Twenty single donor and thirty six random donor platelets were examined. All platelet concentrates were taken on the fifth day of donation. Microbial contamination was detected by automated culture and PCR followed by DNA sequencing.

From the automated culture bottles of the platelet units, 7 out of 56 (12.5%) were positive. PCR and sequencing detected 10 out of 56 samples (17.9%) including those detected by culture. All positive samples were collected by the random donor method and all apheresis platelet units were negative. *Staphylococcus epidermidis* was the most frequent isolate, it was found in 3 (5.4%) units by both methods. One (1.8%) of each of *E. coli*, *Klebsiella*, *Staphylococcus aureus* and *Acinetobacter* was isolated by culture. While by PCR 3 (5.4%) *E. coli* and 2 (3.6%) *Klebsiella* were detected. Each organism was isolated alone in one single platelet unit (Table 2). A highly significant association was detected between the results of platelet units' culture and that of PCR and sequencing ($P < 0.001$) (Table 3). Regarding antibiotic susceptibility testing, all the isolated strains showed different patterns of sensitivity. All *S. epidermidis* and *S. aureus* were methicillin resistant and *Acinetobacter* was multi-drug resistant.

The diagnostic test performance for detection of all organisms isolated by PCR and DNA sequencing in comparison to automated culture (gold standard) was 100% sensitivity and 93.9% specificity. Three false positive and no false negative cases were detected.

The sensitivity and negative predictive value of PCR for the detection of any of the isolated organisms in reference to the automated culture was 100%. The sensitivity and specificity of each of *Staphylococcus epidermidis*, *Acinetobacter* and *Staphylococcus aureus* were 100% as regards the results of PCR versus culture as shown in Table 4.

The present study discusses the bacterial contamination in platelet concentrates and the most frequently isolated bacterial microorganisms. It evaluates PCR and DNA sequencing in comparison to platelet culture technique (gold standard) for the detection of bacterial contamination in platelet concentrates (Yomtovian *et al.*, 2006; Korsak, 2012).

In the current study, automated culturing was done for each of the randomly selected 56 platelet concentrates on the fifth day of donation with a resultant bacterial contamination rate of 12.5% by automated culture and 17.9% by PCR. All positive PCs for bacterial contamination were collected by the random donor method. Similarly Adjei *et al.* (2009) in Ghana reported 9% prevalence of bacterial contamination in PCs. Much lower rates were reported, 0.81% by Claeys and Verhaegle (2000) and 0.033% by Munksgard *et al.*, (2004) and Blajchman *et al.*, (2005). All of studies used the automated culture technique for diagnosis. Countries that perform prospective testing reported variable prevalence of bacterial

contaminated PCs from 0.08% to 0.7% depending on technology, testing protocols and additional intervention methods (Dreier *et al.*, 2007; Canellini *et al.*, 2010; Martinez *et al.*, 2010). The high detection rate in our study may be related to the random donor method of platelet collection and the time of sampling of the PCs on the fifth day of collection. Martinez and coworkers (2010) reported that 89% of the contaminated PCs detected were multi-donor units. Johns Hopkins University demonstrated by culture a reduction in the septic transfusion reactions when switching to single-donor apheresis only, from 7.45 / 100,000 platelet transfusions (1987-1998) to 2 /100,000 platelet transfusions (2004-2007), a 69.7% reduction (Ness *et al.*, 2001; Fuller *et al.*, 2009). In Japan, Vienna, some centers in France and USA, only apheresis platelet concentrates are used. Apheresis platelets are preferred due to the reduced incidence of platelet refractoriness and donor exposure. However, the limited availability of apheresis donors and the considerably greater costs limit the availability of their use (Leitner *et al.*, 2007).

Timing for detection of bacterial contamination in PCs is very critical. Too early sampling may lead to falsely sterile results, as the number of the initially contaminating bacteria is usually very low. So sampling is recommended after the first 24 hours of preparation. The estimated clinical sensitivity for Day 1 culture varies between 22% and 40% (Stormer *et al.*, 2007; Rockville, 2012). Longer culture time may be applied for detection of slow growing bacteria as *Propionibacteria* (Canellini *et al.*, 2010). While too late screening up to the fifth day of PCs validity may lead to higher rates of

bacterial contamination (Stormer *et al.*, 2007). With very low density inoculum size (below 1 CFU/mL), some bacteria cannot grow. Some bacteria could be susceptible to self- or auto-sterilization in platelets through killing by preformed antibodies, complement proteins, lysozymes, or lipoproteins in plasma (Ezaki *et al.*, 2007). Storage of platelets, suspended in gas permeable bags for five days on agitators in room temperature, supply optimal conditions for bacterial proliferation. Rapid proliferation of bacteria occurs to reach 10^8 - 10^9 /ml within the 2nd-5th day of storage (Wagner *et al.* 1995; Korsak, 2012). Most patients can tolerate and clear a bacterial load of 10^4 coagulase negative *Staphylococci* (Larsen *et al.*, 2005; Korsak, 2012). Transfusion of fresh units of PCs and antibiotic administration may explain the lack of clinical consequences in the recipients (Martinez *et al.*, 2010).

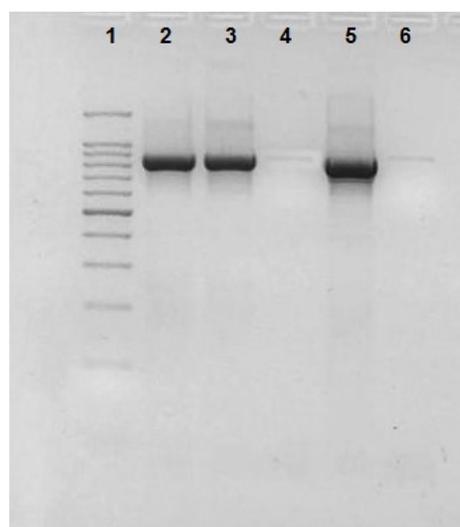
Several researchers in different countries reported that the residual risk of contamination of the cultured platelet apheresis units was still high. Repeated cultures detected almost about half of the contaminated units indicating that the residual risks was in the range of 1/1000 units (AuBuchon, 2012). Repeated culture of outdated, culture negative platelet apheresis units by Dumont *et al.* (2010) and Jacob *et al.* (2011) found 4/6,039 positives (1/1,500) and 9/27,620 (1/3,100) respectively that were missed by Day 1 culturing.

Several approaches to increase the sensitivity of culture for detection of bacterial contamination and to reduce the false negative results were suggested. First is re-culturing on day 3 in order to detect the slowly growing organisms. Increasing

Table.1 PCR primers of 16S rRNA and DNA sequencing

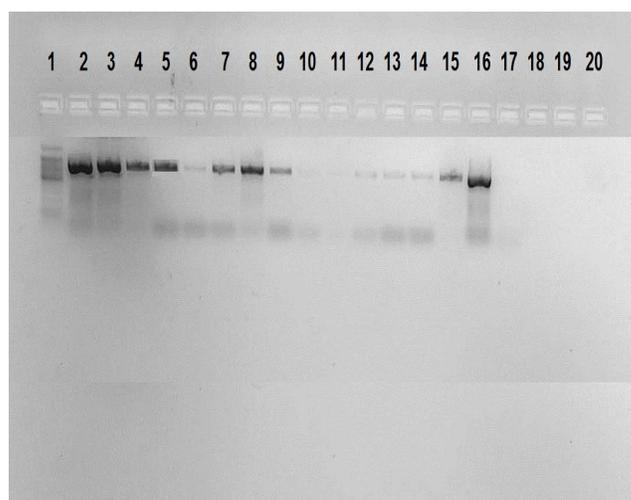
Primers	Sequence
#515 Forward	5'- GTG CCA GCA GCC GCG GTA A-3'
#1390 Reverse	5'- AGG CCC GGG AAC GTA TTC AC -3'
#8-27 Forward	5'- AGA GTT TGA TCC TGG CTC AG -3'
#806 Reverse	5'- GGA CTA CCA GGG TAT CTA AT -3'

Figure.1 Agarose Gel Electrophoresis of the Amplified DNA



Lane 1: 100 bp molecular size marker; Lanes 2, 3: positive samples (400 bp); Lane 4: negative case;
Lane 5: positive control (400 bp); Lane 6: negative control;

Figure.2 Agarose Gel Electrophoresis of the Amplified DNA



Lane 1: 100 bp molecular size marker; Lanes 2, 3, 4, 5, 7, 8, 9, 15: positive samples (400 bp); Lane 4:
negative case; Lane 16: positive control (400 bp); Lane 17: negative control.

Figure.3 Base Calling View of Case no 1 (*Staphylococcus epidermidis*)

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nnnnnnnnn aggatgttgg cttagangca gccacattta aagaaagcgt aatagctcac
tggctogagtc ggcctgocgc gaagatgtca cggggctaaa ccatgaccgc aagctggggc
agcgedacta tgggttggtg ggtaggggag cgttetgtaa gccgttbaag tgggctcttg
anggttgctg gaggtatcar aagtgggat gctgacataa gtaacgataa tgggggtgaa
aaacccgcag gccggagagc caagggttcc tgtccaacgt taatcggggc aggggtgagtc
jacccctag gccagggccga aaggcgtagt ccatcgggaa caggttaata ttoctgtact
tgggtttact gcgaaggggg gacggagaag gctatgttgg cggggcgaag gttgtccogg
tttaagcatg taggcggatq ntccangtar atcccgaacg ttaytaacgc tggggtgtga
tgacsaggca ctacgggtgc gaadtgacaa atgcccctgc tccaggaaag gcctctaagc
atccaggrac atcaaatcgt accccaasac gaccacaggtg gtcaggtaga gaataccaag
gcgcttgaga gaactcgggt gaaggaadta ggcaaaatgg tcccgttaact tggggagaaq
gcaugccgat ggtcaagtga gtagacttgc catggagctg aatcaytey aagatacuaq
ctggctgca ctgtttatta aaaaatcagc actgtgcaaa gacgaaagtg gaggtataag
gtgtgacgoc tggccgggtgc cggaaaggtta attgatgggg ttatcctcac ggaagaagtc
tggatgaaq ccccggtaaa cggggcggct aactataaoc gtcctaaggc agcgaadtc
ctctcgggn nnnnnnnnn nnn
    
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Table.2 Results of platelet unit culture and PCR and sequencing

	Blood Culture No. (%)	PCR No. (%)
Negative cases	49 (87.5%)	46 (82.1%)
<i>Staphylococcus epidermidis</i>	3 (5.4%)	3 (5.4%)
<i>E. Coli</i>	1 (1.8%)	3 (5.4%)
<i>Klebsiella</i>	1 (1.8%)	2 (3.6%)
<i>Staphylococcus aureus</i>	1 (1.8%)	1 (1.8%)
<i>Acinetobacter</i>	1 (1.8%)	1 (1.8%)
Total	56 (100%)	56 (100%)

Table.3 Association between culture and PCR results

		CULTURE		Total No. (%)	
		Positive No. (%)	Negative No. (%)		
PCR	Positive	No. (%)	7 (12.5%)	3 (5.4%)	10 (17.9%)
	Negative	No. (%)	0 (0%)	46 (82.1%)	46 (82.1%)
Total Count		No. (%)	7 (12.5%)	49 (87.5%)	56 (100%)

Table.4 Diagnostic validity test for PCR versus culture

Organism	TN No.	FN No.	FP No.	TP No.	SP %	SN %	P- %	P+ %	Efficacy %
<i>S. epidermidis</i>	53	0	0	3	100	100	100	100	100
<i>Acinetobacter</i>	55	0	0	1	100	100	100	100	100
<i>S. aureus</i>	55	0	0	1	100	100	100	100	100
<i>Klebsiella.</i>	54	0	1	1	98.2	100	100	50	98.2
<i>E.Coli</i>	53	0	2	1	96.4	100	100	33.3	96.4
Any organism	46	0	3	7	93.9	100	100	70	94.6

TN: True Negative; FN: False Negative; FP: False Positive; TP: True Positive; SP: Specificity; SN: Sensitivity; P-: The predictive value for a negative case; P+: The predictive value for a positive case

the volume cultured and using the rapid detection tests just before releasing the units to a patient for transfusion or to repeat testing every day were suggested methods to detect any contamination that would be missed by Day 1 culture. The FDA and the AABB are paying attention to the requirement to perform additional testing beyond Day 1 culture for apheresis platelets (Rood *et al.*, 2008; Eder *et al.*, 2009; Souza *et al.*, 2012).

In this study, *Staphylococcus epidermidis* (3 (5.4%)) was the most frequent microorganism isolated from positive samples by culture followed by *E. coli*, *Klebsiella*, *Acinetobacter* and *Staphylococcus aureus* (1.8% each). While by PCR 3 (5.4%) *E. coli* and 2 (3.6%) *Klebsiella* were detected. Each isolate was separated in a single PC sample. In 2001, Kocazeybek and coworkers reported that microorganisms, which were true contaminants, were mostly *Staphylococcus epidermidis*. They used BACTEC 9050 device for culturing platelet concentrates. Similar results were reported by Katayama *et al.* (2003), Larsen *et al.* (2004), Larsen *et al.* (2005), Yomtovian *et al.* (2006), Martinez *et al.*, (2010), and Jacob *et al.* (2011). Also, Ramirez – Arcos and coworkers (2007) reported coagulase negative *Staphylococci* as the most frequently isolated organism. In addition, two apheresis platelet units contaminated with gram-negative bacteria (*Serratia marcescens* and *Salmonella spp.*) were missed by BacT/ALERT system and transfused causing severe transfusion reactions. The recipient of the unit contaminated with *Serratia marcescens* died.

Several researchers in different countries reported that *S. epidermidis* represents 50% of all bacteria contaminating platelet

concentrates and 25% of bacteria causing complications after PCs transfusion. *Salmonella choleraesuis*, *S. aureus*, *Serratiam arcenscens*, *Bacillus cereus*, *S. viridans* and other bacteria represents 13.5%, 9.6%, 9.6%, 3.8%, 5.8% and 36.5% of bacteria causing complications respectively. Gram negative bacteria are usually associated with rapid course of disease (Korsak, 2012).

Wagner and Eder (2007) reported that *Staphylococcus* organisms proliferate slowly and sometimes exhibit lag times in deliberately inoculated platelet components and are the most frequently isolated organisms implicated in clinical sepsis cases associated with false-negative bacterial culture results.

Slowly growing microorganisms like *Propionobacterium spp.*, which need four to six days to be detected in the anaerobic culture bottles, will not be suitable to be screened by culture as the shelf-life of platelets is five days. Although *Propionobacterium acnes* is considered to be of no or only low clinical significance, three clinical incidents of contamination of platelet concentrates with *Propionobacterium acnes* has been reported by Schneider *et al.* (2000).

Bacteria belonging to the normal skin flora adhere firmly to human hair despite skin antisepsis. These results indicate the importance of observance of the strict compliance to the phlebotomy rules and careful antisepsis of the phlebotomist's hands and the skin of the donor during donation (Motoyama *et al.*, 2008; Korsak, 2012).

The three isolates of *Staphylococcus epidermidis* and *Staphylococcus aureus*, which are members of the skin flora, were

methicillin resistant. *Acinetobacter* isolate was also multi-drug resistant. These points at the need of strict implementation of infection control measures inside the blood bank to decrease the risk of hospital acquired infection especially with multi-resistant drug resistant strains. Similarly Adjei *et al.* (2009) (Ghana) reported that most of the isolated organisms from the blood products in their study were resistant to the used antibiotics in their country.

In the present study, all the positive samples by automated culturing were positively detected by PCR and DNA sequencing. Additional three samples were positive by PCR only and not by culture. The PCR assay had a sensitivity of 100% and a specificity of 93.9%. Mohammadi *et al.*, (2005) compared broad-range real-time 16S rRNA PCR with automated culturing. Positive samples for the presence of bacteria were 0.83% by both methods. Although automated culturing of PCs is considered the gold standard in most blood centers, the PCR assay had almost the same sensitivity as culture.

The automated culture of PCs is very sensitive and can detect reliably bacteria as low as 10 CFU/mL and in many cases to ≤ 5 CFU/mL. But because detecting bacteria from PCs depends on the type and concentration of bacteria and the duration of storage. This leads to detection of positive culture after PCs being transfused to the patients. However, false negative results in automated culture occur due to the low initial load and the slow growers of bacteria (Moriyama *et al.*, 2008; Rood *et al.*, 2008).

Genetic methods as PCR for detection of bacteria in PCs are rapid and very highly sensitive; 10 CFU/container by Schmidt *et al.* (2006) and 22 to 29

CFU/mL for gram-negative and gram-positive bacteria respectively by Rood *et al.* (2008). Detection of bacterial 16S rDNA lasts few hours, so it may be performed before each transfusion making the duration of PCs storage more flexible. The disadvantage is it detects both alive and non-viable bacteria (Mohammadi *et al.*, 2005; Korsak, 2012).

An explanation of the reduced specificity of PCR as compared to culture could be due to the fact that, broad-range 16S rRNA PCR assay could become a source of false-positive results if no sufficient measures to prevent contamination are taken. These include the use of separate rooms for sampling platelet concentrates, for nucleic acid extraction and for setting up PCR procedures. Other sources of contamination with bacterial DNA like PCR enzymes and extraction reagents which must also be precluded. In addition, the use of dedicated devices and disposables needed during the whole procedure of extraction, amplification and detection may greatly increase the costs of the assay. Accordingly, it might be recommended to conduct a cost-benefit analysis (Reesink *et al.*, 2008).

Many methods for detection of bacterial contamination in PCs before transfusion have been developed, ranging from simple inexpensive to sophisticated ones. The selection of a method depends on the expense, sensitivity, performance time and the safety to avoid additional contamination of PCs during processing. Replacing culture by PCR for nucleic acid amplification is suggested for its high sensitivity, rapid detection and standard technology (Muller *et al.*, 2008).

Conclusion and Recommendations

In conclusion, the regular sterility testing

of the platelet concentrate units is mandatory. Although automated culture technique is considered the gold standard method for diagnosing bacterial contamination in PCs, it necessitates early sampling and very large sample volume to improve the chance of detection. In addition; slow growing bacteria are always missed. Use of the highly sensitive PCR with its short turnaround time is of great value to decrease the risk of post-transfusion sepsis especially for the prerelease testing just before actual transfusion. Also adequate infection control measures including skin preparation of the venipuncture site of the donor arm is highly recommended as the most frequent isolate was *Staphylococcus epidermidis*. Use of single donor apheresis platelets only as all the contaminated platelet units detected in our study were random donor platelets. Finally it may be very important to develop assays that detect bacterial mRNAs in platelet concentrates as the 16SrRNA PCR assays do not discriminate between viable and non-viable bacteria.

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