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ORIGINAL ARTICLE

Bacterial Contamination of Stored Blood and Blood Components Ready for Transfusion at the Blood Bank

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ABSTRACT

Background: *Contamination of donated blood is considered a serious health problem. The term (Bacterial contamination) is the existence of bacteria in the blood or its products that are collected in blood bags and stored for transfusion. Blood bags prepared for transfusion should be free from micro-organisms. Blood collection and processing must be done under complete aseptic conditions and techniques. However, sources of the donated blood contamination may be endogenous (the source of contamination is from the donors) or exogenous source (during the collection and processing* ***Objectives:*** *This study aimed to estimate the rate of contamination, and identify the types of micro-organisms detected in the blood products prepared for transfusion and to detect antimicrobial susceptibility (antibiotic sensitivity tests)) of contaminant agents in blood of blood bank bags.* ***Methodology:*** *After adoption of Local Institutional Ethical Committee of Menoufia University Hospital, this study was established at the blood bank of Menoufia University Hospital, Egypt. Over eighteen months from April 2018 to October 2019. One hundred donor's blood samples were withdrawn for culture to detect bacterial growth. In addition, the antibiotic sensitivity tests of the growing micro-organisms were performed.* ***Results:*** *From the 100 sample donor blood, 12 blood bags (12%) showed isolates of different bacteria. Eight of the isolated bacteria were Gram-positive cocci representing 66.7%.* ***Conclusion:*** *The predominantly isolated bacteria were Coagulase-negative Staphylococcus and Staphylococcus aureus. Staph aureus was resistant to ampicillin and cotrimoxazole by susceptibility tests.*

INTRODUCTION

Bacterial contamination of transfusion products is a longstanding problem. The existence of bacteria which survive in the blood or its products that are collected in blood bags and prepared to be transfused is known as bacterial contamination of transfusion products (donated blood)[1]. Blood bags prepared for transfusion should be sterile, without microbial contaminants[2]. This blood should be collected and processed under aseptic conditions and techniques by using modern and improved materials for phlebotomy, donation, collection, separation of component, refrigeration and freezing[3]. There are two routes of contamination either endogenous (the source is the donor) or exogenous (contamination can happen during the withdrawal and preparation route [4,5]. Bacterial contamination of transfused blood products is one of the common causes of mortality from transfusion.

Detection of bacteria in the stored blood can be done using different techniques[6]. The existence of bacteria that survive in the RBCs may produce gas, resulting in unusual air bubbles. This results in a pink to red discoloration that could be noticed in the supernatant[7]. Bacterial contamination of the different blood products (packed red blood cell, fresh frozen plasma, and platelets) has been observed to cause a severe problem in transfusion therapy in the past two decades and is the second only to ABO-mismatch in causing transfusion complications leading to death.[8]

Contamination of the blood products with bacteria is being considered as a main risk during withdrawal. Several factors in different studies showed that: causes of contamination of withdrawn blood with bacteria are due to inadequate disinfection and bad storage[9].

Many studies reported higher levels of contamination in the stored blood products in the developing countries than in developed countries[10].

Sources of bacterial contamination include: donor bacteremia, contamination during the whole blood collection procedure, contamination of collection pack, and contamination during processing procedure. In some cases, this contamination was because of donation in the window phase of the infection when the numbers are too low for detection. Most often the blood gets contaminated with bacteria at the time of bleeding of donors .The blood is then screened for agents other than the bacterial contaminant and then stored and used at

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later date. This period of storage may act as an incubation period for these low numbers to proliferate before it is transfused. After transfusion, hypotension, shock and collapse are common complications[11]. Tooth repair may cause transient bacteremia by *staph aureus* if tooth repair was two hours before donation.

Methods used for detection of bacteremia in transfused blood should be simple and rapid. Ordinary and automated culture are sensitive but require long time. Other than automated culture (gold standard method) there are immunological, molecular and biochemical methods which are highly sensitive.

The aim of the study was to determine rate of prevalence of bacterial contaminations and antimicrobial susceptibility pattern of contaminants in stored blood and blood components.

METHODOLOGY

Specimens

A specimen obtained from blood bags. Large quantities of blood are donated to the blood bank and from campaigns then processed for separation of the blood component. Such donated blood for transfusion formed the study cohort. Donor blood that tested positive for transfusion transmissible infections according to the protocol of blood bank (HIV, HBV, HCV, and Syphilis) was not included in our study. also expired blood and blood that showed plasma turbidity or fibrin clots were not included.

Sample collection and testing

One hundred blood bags have been chosen unintentionally. The samples were taken from the cords of bags after careful disinfection of the puncture site by swabbing the puncture sites with alcohol and leaving them to dry.

Using a sterile syringe after disinfection with tincture of iodine, blood could be withdrawn and delivered into a sterile blood culture bottle. These sample broths would have been incubated at 37ºC for 15 days.

These bottles were sub-cultured by sterile loops on blood agar, MacConkey agar and Chocolate agar plates.

The culture plates had been incubated aerobically while the chocolate agar was being incubated anaerobically.

Identification of bacterial growth was done by morphology of colony, biochemical reactions and gram staining.

Coagulase test was done to differentiate *staph aureus* from coagulase negative staph (CONS).

Antibiotic susceptibility testing has been performed in accordance to the parameters of the Clinical Laboratory Standard Institute. The susceptibility of the isolates to antimicrobial agents was examined by an agar diffusion method on Muller- Hinton agar (Oxoid, UK) using antibiotics impregnated paper discs s (Oxoid, UK), containing the antibiotics with concentrations as shown below: Ampicillin 10mg, Gentamicin 10µg, Cotrimoxazole 25µg, Cefuroxime 30µg, Penicillin 1.5IU, Tetracycline 30µg, Erythromycin 5µg, Cloxacillin 1.5IU Ceftriaxone 30µg, and Chloramphenicol 30µg. This method is consistent with the procedure described by the Clinical and Laboratory Standards Institute (CLS I).

Statistical Analysis:

SPSS 18 (Statistical Package for Social Science) (Chicago, Inc, Illinois) has been used to tabulate and statistically analyze the collected data. Data were entered as numerical or categorical, as appropriate. Two kinds of statistics were performed

- Descriptive statistics, in which quantitative data were expressed in the mean, the standard deviation of the mean (x ± SD), and the standard error (SE).
- Qualitative data which were expressed in number (frequency), and percentage.

Analytical statistics had been done by using Chi-square test and Fisher exact test to measure the association between qualitative variables as appropriate. Also, quantitative data with normal distribution in two groups were evaluated with the student (t-test) while that which is not normally distributed was evaluated by Mann-Whitney test (nonparametric test). The level of significance used was 95% so P (probability) value of >0.05 means insignificant differences, the P-value of <0.05 means statistically significant differences and the P-value of <0.001 means highly significant differences.

RESULTS

From the 100 refrigerated donor blood samples, 12 blood bags showed the presence of isolates of different bacteria. Eight of the isolates were Gram-positive cocci representing 66.7%. (Table 1, Fig.1)

Table 1: Blood culture results

Blood culture	NO	%
Negative cases	88	88%
Coagulase-negative staph	5	5%
Corynebacterium diphtheroids	1	1%
E. Coli	1	1%
Klebsiella pneumonia	1	1%
Staphylococcus aureus	3	3%
Acinetobacter spp	1	1%
Total	100	100%

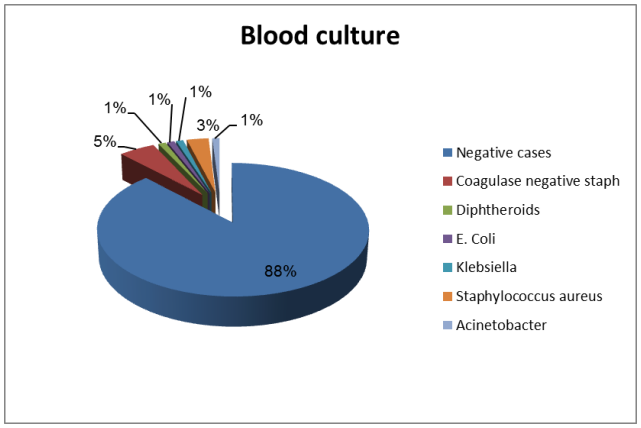

Fig. 1: Percentage distribution of bacterial isolates from donor blood

Five (41.7%) of the gram-positive cocci were detected to be coagulase-negative staphylococci and 3 (25%) were *Staph. aureus*. There were 4 (33.3%) isolates, one was gram-positive rods, and identified as *Corynebacterium diphtheroids*. There were three isolates which were gram negative rods; one was identified as Escherichia coli, the other one was *Klebsiella* pneumonia and the last one was identified as gram negative cocobacilli *Acinetobacter*. (Table 1, Fig.1)

Detection of the susceptibility of these organisms to the antibiotic sensitivity tests revealed that all the twelve organisms isolated were sensitive to Amikacin, but only 10% of the coagulase negative Staphylococci were sensitive to Co-trimoxazole and 50% were sensitive to Tetracycline. Cefuroxime, Gentamicin, and Ciprofloxacin have higher sensitivities with values of 80%, 100%, and 60% respectively. Only the gram positive isolates tested against Erythromycin and Cloxacillin; were coagulase-negative Staphylococci and had sensitivities of 30% and 65% respectively. *Staphylococcus aureus* have been sensitive to Ampicillin and Cotrimoxazole discs.

Table (2): The percentage sensitivity pattern of the various organisms isolated, CNS = Coagulase negative staph

	Gram Positive cocci		**Gram Positive rod**	**Gram Negative rod**		**Gram Negative coccobacilli**
	Coagulase-negative staph	***Staphylococcus aureus***	***Corynebacterium diphtheroids***	***E. Coli***	***Klebsiella pneumonia***	***Acinetobacter spp***
AMP	0	0	0	0	0	0
AK	100	100	100	100	100	100
COT	10	0	0	100	0	0
CPR	60	45	100	100	100	80
CRX	80	71	100	100	100	75
CXC	65	71	0	0	0	0
ERY	30	100	50	0	0	0
GEN	100	100	100	100	100	90
PEN	0	100	100	0	0	0
TET	50	40	0	0	0	0
CLI	80	100	100	100	100	90

AMP-Ampicillin; AK-Amikacin; COT-Cotrimoxazole; CPR-Ciprofloxacin; CRX- Cefuroxime; CXC- Cloxacillin; ERY-Erythromycin; GEN-Gentamicin PEN-Penicillin; TET-Tetracycline. CLI; clindamycin.

DISCUSSION

Sources of bacterial contamination include: donor bacteremia, contamination during the collection procedure, contamination of collection pack, and contamination during processing procedure.

Detection of a small number of bacteria in the blood which only lead to transient bacteremia on transfusion[12] or as a result of coverage of antibiotics[12-14] which masks the signs and symptoms due to extensive use, long storage time gives a chance for bacteria to proliferate. Platelets which live at 22ºC allow also survival of bacteria if contaminated. This may cause a febrile non haemolytic transfusion reaction (FNHTR).

In the present study, *Staph epidermidis* had been representing more than 40% of all the bacteria contaminating blood concentrates. Several researchers have noticed that, 25% of bacteria causing complications after platelet concentrate transfusion. *Salmonella choleraesuis, S. aureus, Serratia marcenscens, Bacillus cereus, S. viridans*, and other bacteria represent 13.5%, 9.6%, 9.6%, 3.8%, 5.8% and 36.5% of bacteria causing complications respectively.

Gram negative bacteria have been noticed to be associated with a rapid course of the disease[17]. Wagner and Eder[18] reported that *Staphylococcus* organisms proliferate slowly and lag times may be exhibited 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 micro-organisms like *Propionibacterium* 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 *Propionibacterium acnes* is considered to be of no or only low clinical significance, three clinical incidents of contamination of platelet concentrate with *Propionibacterium acnes* has been reported by Schneider et al.[17]. Bacteria belonging to the ordinary skin flora adhere firmly to human hair despite skin antisepsis. These results indicate the importance of following the strict compliance to the phlebotomy rules and careful antisepsis of the phlebotomist's hands and the skin of the donor during donation[15,18]. The three isolates of *Staphylococcus epidermidis* and *Staphylococcus aureus*, which are members of the skin flora were methicillin-resistant. *Acineto*bacter isolate was also multi-drug resistant.

Staphylococcus epidermidis belonges to coagulase negative *staph.contamaination by staph epidermidis* is almost from infected skin during phelebotomy

Staph aureus is normal inhabitant found on skin and mucous membrane showing asymptomatic benign colonization, enter blood via skin trauma or puncture[17]

Gram negative bacteria endotoxins have fatal effects when a great load of bacteria is amenable to transmission during transfusion.

These points are in need for a strict application of infection control policies inside the blood bank to reduce the incidence of hospital-acquired infection especially with multi resistant drug-resistant strains. Similarly, Adjei et al.[18] (Ghana) reported that most of the micro-organisms isolated from the blood products in their study have been resistant to the used antibiotics in their country.

To reduce blood contamination, proper skin disinfection of donors is mandatory.[13,20,21]. Other causes of contamination other than skin contaminants are the problems induced by the hematogenous parasites as the Malaria, and other pathogens as *Yersinia enterocolitica* and the Salmonella sp in asymptomatic donors. The results obtained in this study are mostly skin-associated organisms or indicators of fecal contamination organisms and are often considered related to the withdrawal procedure[22], or the inoculation procedure for culture[23]. Bacteremia caused by coagulase-negative staphylococci, including other cutaneous microbiota like diphtheroid, is difficult to demonstrate as a 'true pathogen', and therefore, it can be ignored in routine diagnosis. Bacteremia caused by these organisms can induce severe complications in immuno-compromised patients[20,21] such as premature babies and newborns.

In our study, the isolates obtained showed a variable response to the antimicrobials impregnated in Muller-Hinton agar in sensitivity tests. While all isolates were sensitive to Amikacin, only 14.2% and 28.5% of the coagulase-negative staphylococci isolated were sensitive to Cotrimoxazole and Tetracyclines respectively[24,25]. There are great risks of transfusing the contaminated blood and transfusing blood with multidrug-resistant strains of bacteria which may worsen the plight of the already sick and the immunocompromised.

Another explanation for the high resistance of isolated bacteria is the ease of procuring antibiotics over[26,27,28]

CONCLUSION

It is important to note bacterial contamination of the stored blood preserved for transfusion is a significant problem with multiple causes. Most are related to contamination ofthe blood bags is usually the result of improper skin disinfection during blood collection. We strongly recommend that infection control rules must be strict and strongly supervised all the time. However, if the source is occult bacteremia of the donor, careful selection and questionnaire fulfill will reduce the problem.

Accordingly, avoidance of contamination during collection necessitates proper sterilization during phlebotomy and processing.

Recommendations

We recommend that national surveillance programs should be established to study the rank of blood bank blood contamination with bacteria, to establish national levels of contamination. Continued surveillance of donor blood contamination is recommended. Therefore, susceptibility patterns and a larger sample size of isolates will assort a more obvious reply to the issue of donor blood contamination.

Screening of blood by automated blood culture is suggested as a method of pre- transfusion detection of bacteria.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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