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Original Article

Retrospective Study of the Prevalence of Bacterial Contamination of Platelet Concentrates in Morocco

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ABSTRACT

Objective: This retrospective study was conducted using data from the 2005 to 2012 archives at the Laboratory of Bacteriology Serology and Hygiene of Ibn Sina University Hospital Center in Rabat, Morocco. Its aim is to determine the prevalence of bacterial contamination of platelet concentrates produced by the Regional Center for Blood Transfusion of Rabat, after the implementation of the bacterial quality control of the platelet concentrates.**Methods:** A total of 3898 platelet concentrates, obtained after disinfection of the sampling site and diversion of the first milliliters of the blood donation, were cultured to study bacterial contamination between 2005 and 2012. The obtained bacterial colonies are tested by Gram staining and orientation tests such as: catalase, coagulase, oxidase, as well as the biochemical gallery for identification. This retrospective study was conducted over a period of six months using data from the archives of the Laboratory of Bacteriology Serology and Hygiene of Ibn Sina University Hospital Center in Rabat, Morocco. **Results:** This retrospective study concluded that the prevalence of bacterial contamination in platelet concentrates is 0.44 %. It also showed the presence of the following bacteria species: coagulase-negative *Staphylococcus species* and *Bacillus* sp each at 29.41%, *Staphylococcus aureus, Streptocoque, E.coli* and *Enterobacter* each were found at 5.9%. Other bacilli's grams negative were present at 17.65 %. **Conclusion:** Even though, the prevalence of bacterial blood contamination found in this study was low, blood transfusion in Morocco has a long way to go before reaching the standards set and commonly accepted in developed countries.

Key words: Bacterial contamination, Blood donor, Blood safety, Morocco, Platelet concentrates.

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INTRODUCTION

Bacterial contamination of blood products is still the largest residual source of transfusion transmitted diseases.[1] The prevalence of bacterial contamination of blood was found to be between 0.2% and 4 %.[2,3] The clinical consequences of transfusing bacterially contaminated blood range from minimal or no reaction to fatal septic shock and death. The platelet concentrates (PCs) were found to be the most contaminated.[4,5] The improvement of the disinfection procedures and the use of the diversion of the first milliliters of donor blood, significantly reduce contamination risks. Many research studied the effect inactivating transfusion-transmitted pathogens to improve blood,[6,7] however the bacterial contamination of platelet concentrates remains worrying.[4] Especially in resources limited countries, where the risk of transfusion-transmitted infections is still considerable.[5] The most beneficial bacterial preventative measures taken by the Rabat blood center in Morocco, was the implementation of this bacterial quality control on PCs. Over the last 10 years blood safety in Morocco, achieved a great deal of improvement.[8]

This study is the first of its kind in the country that monitored the prevalence of bacterial PCs contamination on day 2 after blood collection. For this purpose, a retrospective study, to determine the prevalence of bacterial contamination of PCs produced by Regional Center for Blood Transfusion (CRTS) of Rabat was conducted.

MATERIALS AND METHODS

In Morocco, blood donors' recruitment follows the Ministry of Health recommendations.[9] Only consenting and voluntary blood donors are eligible to donate blood, the donors have to be healthy, aged between 18 and 65 years old and weigh at least 50 kg. The interval between whole blood donations is at least 8 weeks, with a maximum of three donations for women and five donations for men per year. All potential donors are interviewed by a medical doctor to discuss donor's health history. Each donor receives a brief examination including temperature, pulse and blood pressure. We followed Moroccan law for the collected volume.[9,10]

Since December 2005, Laboratory of Bacteriology Serology and Hygiene (LBSH) of the University Hospital Center (CHU's) Ibn Sina of Rabat, was tasked to run bacterial quality control of PCs prepared by the CRTS. This blood transfusion center on the other hand, was supposed to send a random number of PCs (from 4 PCs to16 PCs per week) to LBSH of the CHU's Ibn Sina of Rabat, Morocco.

Samples

From the early days of the collaboration until December 2012, 3898 PCs samples (less than 2 days from the collection date) sent by the CRTS were screened. In this retrospective study, the bacteriological results from the LBSH of the CHU's Ibn Sina of Rabat files of the 3898 PCs samples were analyzed.

Bacteriological detection methodology

At the CHU laboratory, every PCs are disinfected for at least one minute with a disinfectant (70% concentrated Alcohol),[3] the bacterial culture was done aseptically from PCs samples following the World Health Organization (WHO) recommendations.[11]

The bacterial cultures broths were incubated in aerobic atmosphere at 37°C for 7 days and were daily controlled for any possible signs of bacterial growth (turbidity, color, voile…). In case of a positive sign, the corresponding bacterial cultures broths were systematically cultivated aseptically in the five media (chocolate agar, eosin methylene blue agar medium, agar chapman, agar cetrimide and Listeria Palcam agar). These media were pre-controlled to ensure reproducibility and avoid any false negatives or false positives due to contamination. Gram staining and biochemical identification were subsequently performed on every colony growing in the seeded media.

The bacteriological analysis was then followed by orientation tests such as: catalase, oxidase and coagulase. Biochemical identification by ordinary gallery was performed and was confirmed by the API systems (bioMérieux). In case of any bacterial growth, the CRTS was immediately notified to run hemovigilance procedures on fresh frozen plasma and Red cell packs from the same blood donor.

Statistical data analysis

Microsoft Excel 2010 was used to sort and organize the data. After this data was analysed using, IBM SPSS Statistics version 20. Qualitative data were expressed as percentage. Every value of $p< 0.05$ was considered statistically significant.

RESULTS

Bacterial screening of 3898 PCs (less than 2 days from the collection date) analyzed over a period of 7 years, showed that 17 samples (0.44%) had bacterial proliferation. Year to year prevalence showed a decrease (1.79% to 0%) since the first year of the exploration [Table 1]. Indeed, in the first year, out of the 919 PCs samples controlled, 11 (1.20%) tested positive [Table 2]; on 2005 (December) 1 PCs out of 56 was contaminated by *Bacillus sp* and in 2006, 10 PCs samples out of 863 (1.16%) were found to be contaminated. In this year, 5 types of bacteria were detected, namely: *Bacillus. sp* in 2 PCs, bacilli gram negative (BGN) in 5 PCs, coagulase-negative *Staphylococcus* in 2 PCs, *Staphylococcus aureus* in 1 PCs and *Streptocoque* in 1 PCs [Table 1]. The number of tested positive samples quickly decreased in the second year (2007) (0.36% and 0%) out of 2137 total PCs tested [Table 1].

The bacteria type found in this retrospective study were cocci, bacilli gram positive and bacilli gram negative as follows: coagulase-negative *Staphylococcus* species and *Bacillus* sp, each in 5 PCs out of 17(29.41%) and other bacilli gram negative in 3 PCs out of 17 (17.65 %). *Staphylococcus aureus, E. coli*, *Streptocoque* and *Enterobacter,* each in one PCs out of 17(5.9%), were found as shown in [Figure 1].

DISCUSSION

Best practices in health care are lead to successful clinical goals.[12] Similarly, to reach blood safety, transfusion processes need to be reviewed and controlled at all steps specially blood unit's products as platelets. The PCs which are supposed to be stored at (22°C ± 2°C), are more concerned about bacterial contamination than other blood components.[4] This is the first study in Morocco that monitored the prevalence of bacterial PCs

Table 1: Number and percent of positive PCs and the distribution of bacteria by type per year.

Year	Number of PC sanalyzed	Number of positive PCs	% Positif PCs	Bacteriaspecies				
				Streptocoque	*Bacilli* gram negative	*Bacillus.* sp	*coagulase*-negative *Staphylococcus*	*Staphylococcus aureus*
2005	56	1	1,79	0	0	1	0	0
2006	863	10	1,16	1	5	1	2	1
2007	842	3	0,36	0	0	2	1	0
2008	527	0	0	0	0	0	0	0
2009	220	0	0	0	0	0	0	0
2010	597	1	0,17	0	0	1	0	0
2011	552	2	0,36	0	0	0	2	0
2012	241	0	0	0	0	0	0	0
Total	3898	17	0.44	1	5	5	5	1

Table 2: Number and percent of positive PCs by season and by year.

Year	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Total
Total of PCs	919	842	527	220	597	552	241	**3898**
Total of positifs and (%)	11 (1.20)	3 (0.35)	0(0)	0(0)	1 (0.16)	2(0.36)	0 (0)	17(0.44)
Octobre - Mai	680	561	359	176	497	376	169	2818
Total positive between Oct and May and (%)	2 (0,29)	0(0)	0(0)	0(0)	1(0,20)	2 (0,53)	0	5 (0,18)
Juin - Septembre	239	281	168	44	100	176	72	1080
Positive total between June and September and (%)	9(3,76)	3(1,07)	0(0)	0(0)	0(0)	0(0)	0(0)	12 (1,11)

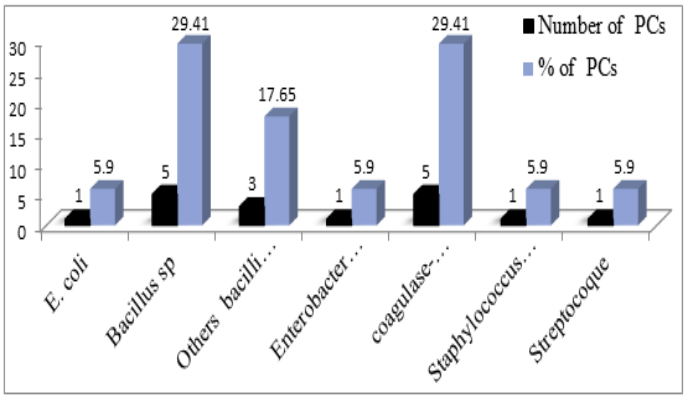

Figure 1: Number and percent of bacteria species isolated in platelet concentrates.

contamination on day 2 after blood collection. Since December 2005, for quality assurance purposes, a collaboration between LBSH of the CHU's Ibn Sina of Rabat and CRTS was made to monitor the bacterial contamination of PCs. Every day, one to 4 PCs samples, aged less than 2 days from the collection of blood, were randomly chosen by the quality control manager from CRTS and sent to the LBSH of the CHU's Ibn Sina of Rabat, for a blind screening of any potential bacterial presence.

This study is based on the exploration of the records and files of the CHU laboratory for the period 2006 to 2012, determined that the overall prevalence of bacterial contamination of PCs during the7 years period was 0.44% PCs. This prevalence is lower compared to the results reported in other studies carried out in many developing countries such as in India,[5] but it remains very high in comparison with the reported bacterial contamination prevalence in developed countries,[2] as United States or France. However, the prevalence found in our study showed a decrease of the platelets' contamination during the years. The variation of the prevalence of PCs bacterial contamination during the 7 years study period is very significant (*p* value less than 0.01).

A great effort has been made to make blood products safer and could explain the prevalence decrease; as the use and the enforcement of PCs bacterial quality control procedures, the implementation of a new version of the best manufacturing practices, the application of the "two-step" disinfection process at the blood donor antecubital fossa, the application of quality management tools as internal audits, surveillance and close follow up for non-conformities and finally, the launch and the implementing of the new hemovigilance law in 2005.

In addition a variation in the prevalence was noticed within each year. In fact, it was observed that more PCs samples tested positive during the summer season. During the latter that starts in Morocco, from early June and spans until mid-October, the total of contaminated PCs samples in this period was higher. As a matter of fact, (1.11%) analyzed PCs were positives, but in the October-May period, (0.18%) analyzed PCs were contaminated.

This higher prevalence (1.11%) during summer could be attributed to the optimal conditions that foster the growth of these bacteria; as a consequence, this will need stringent application of best practices with respect to donor's recruitment and sample preparation to prevent bacterial blood contamination in the first place.

Important bacterial species diversity was observed in this retrospective study. The majority of the bacteria (70.58%), were Gram positive bacteria,[1] while Gram negative bacteria represented (29.41%). These two bacteria types are responsible for the increased incidence of severe sepsis and septic shock as reported by (Babu M, *et al.*)[13] The isolated bacteria in this study, as found in other research were part of the skin flora.[3] Similar bacteria on the antecubital fossa of potential blood donors were found in an early study by our group (Article in press). With the exception of *Staphylococcus aureus* which can causing a serious problems,[14] most of the time, these bacterial species found in this study are generally associated with non-fatal septic transfusion reactions.[15] It is well known that these species do not grow at low temperatures (between 2°C and 6°C) which correspond to the storage temperature of Red cell. They however will under ideal storage temperature (22°C ± 2°C). Under the latter conditions, the proliferation of micro-organisms becomes possible and could pose serious problems to the PCs recipients.

CONCLUSION

Even though the bacterial contamination of donor blood seems to be decreasing, few contaminated PCs samples are still transfused. There are several mechanisms use to inhibit the growth of micro-organisms. Consequently, in the case of resource-limited countries such as Morocco, only avoidance of contamination by rigorous application of blood collection best practices, quality surveillance during blood units preparation, the ultimate and individual visual inspection before delivering blood units and finally by close surveillance of the recipients during and after the transfusion, can keep the fatalities as low as possible.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ABBREVIATIONS

PCs: Platelet concentrates; **CRTS:** Regional Center for Blood Transfusion; **LBHS:** Laboratory of Bacteriology Serology and Hygiene; **CHU's:** University Hospital Center; **WHO:** World Health Organization; **BGN:** Bacilli gram negative.

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