Distribution, origin and contamination risk of coagulase-negative staphylococci from platelet concentrates

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Transfusion-associated bacterial sepsis is the most common microbiological risk of transfusion and is caused mostly by platelet concentrates (PCs). The most frequently identified bacterial contaminants of PCs are coagulase-negative staphylococci (CNS). In order to learn more about the distribution, source and risk of the CNS that are involved in bacterial contamination of PCs, CNS strains isolated during platelet screening were collected and characterized to the species level with three different methods: 16S rRNA and *sodA* gene sequencing, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and amplified fragment length polymorphism (AFLP) analysis. AFLP analysis was also used for the typing of the CNS strains. A total of 83 CNS strains were analysed by sequencing and 8 different CNS species were identified, with *Staphylococcus epidermidis* being the predominant species. MALDI-TOF MS and AFLP analysis confirmed these results to a large extent. However, MALDI-TOF MS could not identify all strains to the species level and AFLP analysis revealed an additional, likely novel, CNS species. The species identified are mainly recognized as being part of the normal skin flora. Typing of the CNS strains by AFLP analysis showed that there was not a unique strain which is significantly more often present during bacterial contamination of PCs.

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INTRODUCTION

Bacterial contamination of blood products is the main infectious hazard of blood transfusion. From all blood products, platelet concentrates (PCs) are at the highest risk because their storage conditions, at room temperature under constant agitation, support bacterial growth. In the Netherlands, the screening of PCs for the presence of bacteria is performed by automated culturing with the BacT/ALERT culture system (bioMérieux) and about 0.37% of all whole blood derived PCs test positive for bacterial growth (de Korte *et al.*, 2006). It is assumed that bacterial contamination of PCs originates primarily from the skin at the time of phlebotomy and less frequently from asymptomatic donor bacteraemia or from contamination introduced during processing of the units. This is reflected in the types of bacteria that are most commonly implicated in these events; the most frequently identified bacterial

contaminants of PCs are *Propionibacterium acnes* and coagulase-negative staphylococci (CNS) (de Korte *et al.*, 2006). *P. acnes* is a Gram-positive, non-spore-forming anaerobic bacterium that is part of the normal skin flora. Adverse transfusion reactions with *P. acnes* are rare (Sazama, 1990; Wagner *et al.*, 1994) and therefore contamination of PCs with *P. acnes* is considered clinically less relevant. However, several adverse transfusion reactions due to PCs contaminated with CNS have been reported (Dumont *et al.*, 2010; Eder *et al.*, 2007; Hsueh *et al.*, 2009; Jacobs *et al.*, 2008; Sazama, 1990; Wagner *et al.*, 1994). In the Netherlands at the Dutch blood bank, Sanquin, most of the CNS strains that are found in the positive BacT/ALERT bottles are only scored as CNS and not identified to the species level. In this study, we wanted to gain more insight in the different CNS species involved in bacterial contamination of PCs and learn more about the source and risk of contamination. For this purpose, 67 CNS strains that were isolated throughout 2007 and 2008 from PCs by screening with the BacT/ALERT system in the Sanquin North-West and South-East regions were collected. In addition, 16 CNS strains from an unrelated population

Abbreviations: AFLP, amplified fragment length polymorphism; CBS, Canadian Blood Services; CNS, coagulase-negative staphylococci; MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; PC, platelet concentrate.

isolated during routine screening of PCs by the Canadian Blood Services (CBS) from 2006 to 2009 were included to see if there was a difference in CNS strains found at blood banks in different countries. The strains were determined to the species level by: the sequencing of ~500 bp of the 16S rRNA gene or ~400 bp of the *sodA* gene, encoding the manganese-dependent superoxide dismutase; by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis as a new and fast alternative to sequencing; and with amplified fragment length polymorphism (AFLP) analysis. Furthermore, AFLP was used for typing to determine whether specific strains are involved more frequently in the contamination of PC.

METHODS

Bacterial strains. A total of 67 CNS strains isolated from positive BacT/ALERT culture bottles were collected from the Sanquin North-West and South-East regions. The strains had previously been cultured from PCs produced at the Sanquin blood bank services according to the screening procedure as described previously (de Korte *et al.*, 2006). Briefly, an aerobic and anaerobic culture bottle was inoculated with approximately 7.5 ml PC per bottle. Inoculation was performed under aseptic conditions provided by a laminar airflow cabinet. Culture bottles were incubated in the BacT/ALERT culture system and positively flagged bottles were sent to certified microbiological laboratories for confirmation and identification. The strains were collected over a period of 2 years. An unrelated collection of CNS strains ($n=16$) that were isolated during routine screening of PCs by the CBS over 3 years was included (strains from 2006 to 2007 were described previously by Greco *et al.*, 2008). At the CBS only aerobic culture bottles are inoculated. All isolates were cultured on blood agar at 37 °C under aerobic conditions. *Staphylococcus saccharolyticus* strains could not be cultured under aerobic conditions and were cultured under anaerobic conditions for 2 days on blood agar plates. *Staphylococcus epidermidis* (ATCC 14490T and ATCC 12228), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus caprae* (DSM 20608), *Staphylococcus capitis* (LMD89.175) and *S. saccharolyticus* (DSM 20359) were used as reference strains to define the windows of similarity for AFLP. *Escherichia coli* (DSM 1576) was used as a quality control of the MALDI-TOF MS.

DNA extraction. Prior to extraction of DNA, pure bacterial colonies from overnight cultures on blood agar plates were suspended in 100 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) and adjusted to match a turbidity of a 0.5 McFarland standard. The suspension was incubated with 10 mg lysozyme ml⁻¹ at 37 °C for at least 1 h. DNA was isolated with the MagNA Pure LC automated extraction system using the MagNA Pure LC DNA isolation kit III (Roche Diagnostics). Prior to isolation, the samples were incubated with bacteria lysis buffer and proteinase K at 56 °C for at least 1 h and subsequently loaded in the MagNA Pure and extracted. Extracted nucleic acids were eluted in 100 µl elution buffer and stored at -20 °C.

PCR amplification and sequencing. The 16S rRNA gene was amplified using universal primers 5'-TGGAGAGTTTGATCCTGGCT-CAG-3' and 5'-TACCGGCTGCTGGCAC-3' generating a ~500 bp amplicon (Hall *et al.*, 2003). The *sodA* degenerate primers *d1* (5'-CCITAYICITAYGAYGCIYTIGARCC-3') and *d2* (5'-ARRTARTAI-CRTGYTCCCAIACRTC-3') were used to amplify an internal fragment, of the *sodA* gene (Poyart *et al.*, 2001). Samples contained 1 × PCR buffer, 0.2 mM each deoxynucleotide triphosphate, 0.5 µM each primer (Invitrogen Life Technologies), 1.5 mM MgCl₂, 1.25 U Platinum GoTaq DNA polymerase (Promega Benelux), and 1 µl bacterial DNA in a total volume of 50 µl. PCR conditions for 16S rRNA

gene amplification were: 95 °C for 2 min; 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s; and a final step at 72 °C for 5 min. PCR conditions for amplification of the *sodA* gene were: 95 °C for 2 min; 35 cycles of 95 °C for 30 s, 37 °C for 60 s and 72 °C for 45 s; and a final step at 72 °C for 5 min. PCR products were purified using ExoSAP-IT (USB). Sequencing was performed using a Big Dye terminator sequencing kit (Applied Biosystems). The sequence products were purified and then subjected to analysis on an ABI Prism 3100 automated DNA sequence analyser (Applied Biosystems). Sequence homology analysis was performed with BioEdit version 7.0.4.1., the basic local alignment search tool (BLAST www.ncbi.nlm.nih.gov) and sequences from culture collection strains (Becker *et al.*, 2004; Poyart *et al.*, 2001) to confirm gene identity.

MALDI-TOF MS. For MALDI-TOF MS, fresh colonies from blood agar plates were used and a thin smear of bacteria was deposited on a FlexiMass TM target and 1 µl α -cyano-4-hydroxycinnamic acid matrix solution (Shimadzu) was added. The sample was co-crystallized by air-drying at room temperature. Measurements were performed on an AXIMA MALDI-TOF mass spectrometer (Shimadzu). Spectra were recorded in the positive linear mode within a mass range of 2000 to 20 000 Da. Each spectrum was accumulated from 1000 laser shot cycles and automatically processed before being exported to SARAMIS (spectral archive and microbial identification system; Anagnos Tec) for the identification procedure.

Strain typing by AFLP. The AFLP protocol, and restriction site-specific adaptor and primer sequences, have been used as described by Vos *et al.* (1995). Briefly, the restriction/ligation reaction mixtures consisted of 10 ng DNA, 1 × T4 DNA ligase buffer, 0.05 M NaCl, 0.5 µg BSA, 1 pmol *EcoRI* adaptor oligonucleotide, 10 pmol *MseI* adaptor oligonucleotide (Eurogentec), 80 U T4 DNA ligase, 1 U *EcoRI* and 1 U *MseI*. All enzymes were purchased from New England Biolabs. After incubation at 37 °C for 3 h, the mixtures were diluted 1:20 in 0.1 × TE buffer. For amplification of the restriction fragments, 5 µl of the diluted mixture was added to 5 µl PCR mixture, which consisted of 1 × PCR buffer (Sphaero Q) 200 µM dNTPs (Promega), 1 U Super Taq (Sphaero Q), and 20 ng *Eco-A* primer and 60 ng *Mse-C* primer. The *Eco-A* primer was fluorescently labelled with carboxyfluorescein (Eurogentec). Amplification was carried out under the following conditions: 2 min at 72 °C; followed by 12 cycles of 30 s at 94 °C, 30 s at 65 °C and 1 min at 72 °C; and then 23 cycles of 30 s at 94 °C, 30 s at 56 °C and 1 min at 72 °C; and ended by a single extension at 72 °C for 1 min. Before analysis on an ABI Prism 3130 sequencer (Applied Biosystems), 2.5 µl each PCR product was added to 22 µl Hi-Di formamide and 0.5 µl GeneScan-600 LIZ size standard (Applied Biosystems). Data were analysed with the GENESCAN analysis software (Applied Biosystems) and the BioNumerics software package, version 5.10 (Applied Maths). Similarity coefficients were calculated with Pearson correlation and dendrograms were obtained by UPGMA clustering. The windows of similarity were defined by analysing staphylococcal reference strains three times in three different runs.

RESULTS

CNS strains from PCs

A total of 83 CNS strains were included in this study; they were isolated from BacT/ALERT culture bottles that were inoculated with PCs and flagged positive within 7 days of culture. The 83 strains were found in the Sanquin North-West ($n=45$) and South-East ($n=22$) region during 2007 and 2008, and at the CBS ($n=16$) from 2006 to 2009. The strains were analysed at the species level by sequencing. For

this, ~500 bp of the 16S rRNA gene of all strains were sequenced and in case of discrepant results, ~400 bp of the *sodA* gene of some strains were also sequenced. 16S rRNA gene sequencing revealed nine different CNS species, with *S. epidermidis* as the predominant group ($n=35$) both in the Netherlands and in Canada (Table 1). However, the *sodA* gene sequencing of representatives of the different groups revealed only eight different CNS species. This was due to the fact that the *S. caprae* strains ($n=4$, initially identified by 16S rRNA gene sequencing) were identified as *S. saccharolyticus* by *sodA* sequencing. In the case of discrepancies between the two sequencing methods, the results from *sodA* sequencing were decisive because the staphylococcal *sodA* genes exhibit a higher divergence than does the corresponding 16S rRNA gene. One CNS strain shared 99.4% identity with the 16S rRNA fragment and 95.6% identity with the *sodA* fragment of *S. capitis* DSM 20326. This strain was included in the *S. capitis* group (Table 1).

The above-mentioned CNS strains were originally cultured from 81 unique PCs. Two independent BacT/ALERT culture bottles contained two different CNS strains each, both of which contained *Staphylococcus warneri* as well as *Staphylococcus pasteurii*. For 6 of the 81 unique PCs, the BacT/ALERT gave a positive result in both the aerobic and anaerobic culture bottle. In only two out of these six cases, both CNS species found in the two culture bottles were still available. In each case the strains from the two respective culture bottles were of identical species (*S. capitis* or *S. epidermidis*). Reculturing of these two PC bags gave a negative result. Confirmatory testing of the initial positive PC bags from the Sanquin North-West and South-East region in this study gave in 14 cases a positive result, in 38 a

negative result and in 15 cases the initial positive PC culture could not be confirmed because the PC was not available for reculturing (Table 1). The strains that were found during reculturing of the initial positive PCs were not available for analysis. All strains obtained from the CBS were classified as true positives, that is, the same micro-organism was isolated during initial and confirmatory testing (Table 1) (Ramírez-Arcos *et al.*, 2007). This definition is different in The Netherlands, where an initial positive PC is regarded as true positive when a micro-organism is cultured from the positively flagged bottle (de Korte, 2003).

MALDI-TOF MS

The CNS strains were analysed by MALDI-TOF MS/SARAMIS and the results were compared to the results from sequencing. All strains, except for *S. pasteurii* and *S. saccharolyticus*, were correctly identified to species level (Table 2). The majority of the CNS strains (53 out of 83) could be determined to species level with an identification confidence of $\geq 99.9\%$, 16 could be determined to the species level with an identification confidence of $\geq 90\%$. One *S. capitis* strain showed an ambiguous result and was identified as *S. capitis/S. caprae* with an identification confidence of $\geq 90\%$. Two CNS strains were misidentified by MALDI-TOF MS; this concerned two *S. pasteurii* strains that were identified as *S. warneri* with an identification confidence of $<90\%$. The two remaining *S. pasteurii* strains could not be identified at all, as well as four of the *S. saccharolyticus* strains. Six of the *S. saccharolyticus* strains could only be identified to the *Staphylococcus* genus level with a low identification confidence between 50 and 55%,

Table 1. Distribution of CNS strains

The strains were found in initial positive BacT/ALERT cultures from PCs in the North-West and South-East districts and from confirmed positive PCs (after reculture) from Canada (CBS). For the Dutch strains, a reculture of the initial positive PC was performed if the PC was available and results are given as positive reculture (+), negative reculture (–) or not available for reculturing (NA). The strains were identified to the species level by the sequencing of ~500 bp of the 16S rRNA gene or ~400 bp of the *sodA* gene. In case of discrepancies between the two sequencing methods, the results from the *sodA* gene sequencing were decisive. *S.*, *Staphylococcus*.

Strain	The Netherlands – North-West district				The Netherlands – South-East district				Canada	Total
	First culture	Reculture			First culture	Reculture				
		+	–	NA		+	–	NA		
<i>S. epidermidis</i>	17	4	8	5	7	1	4	2	11	35
<i>S. capitis</i>	9	4	5		3		2	1	3	15
<i>S. warneri</i>	6		3	3	3		2	1	1	10
<i>S. saccharolyticus</i>	4	3	1		6	1	2	3		10
<i>S. hominis</i>	6		6		1	1				7
<i>S. pasteurii</i>	2		2		2		2			4
<i>S. saprophyticus</i>	1		1							1
<i>S. lugdunensis</i>									1	1
Total	45				22				16	83

which is below the threshold of 70 % confidence considered for a reliable identification. The reason for this lack of identification was that *S. saccharolyticus* was not yet available in the SARAMIS spectra database. In contrast, spectra of *S. pasteurii* strains were available in the SARAMIS database.

AFLP

The CNS strains were analysed and typed by AFLP analysis. For *S. epidermidis*, strains clustering with a similarity between 80 and 100 % were defined as identical CNS strains. For all species, strains clustering with a similarity above 35 % were defined as CNS strains of the same species and strains clustering with a similarity below 35 % were defined as different CNS species. Analysis of all 83 CNS strains according to the cut off values stated above revealed that there were nine different CNS species present. One strain that was identified as *S. capitis* with sequencing and *S. capitis*/*S. caprae* with MALDI-TOF MS did not cluster within 35 % of the other *S. capitis* strains, defining it as a different species by AFLP (strain 08019; Fig. 1). The *S. capitis* strains that were found in both the aerobic and anaerobic culture bottles that were inoculated from one PC (strains 502 and 534; Fig. 2) showed an almost identical band pattern, which made it highly likely that they were identical strains. The *S. epidermidis* strains that were found in the aerobic and anaerobic culture bottles from the same PCs showed a similarity of >90 %, which identified them as identical strains (strains 506 and 546; Fig. 2). Analysis of *S. caprae* and *S. saccharolyticus* DSM (Deutsche Sammlung von Mikroorganismen) reference strains confirmed the *sodA* sequencing results; there were no *S. caprae* strains in the collection and ten *S. saccharolyticus* strains (data not

shown). All CNS strains belonging to the same species from the different populations from the Netherlands and Canada clustered randomly within a group. As an example, the AFLP pattern of *S. epidermidis* is presented in Fig. 2. This indicates that there are no specific clones involved in contamination of PCs in either of the countries.

DISCUSSION

CNS strains are among the most frequently isolated bacteria in PCs (de Korte *et al.*, 2006). In an attempt to gain more insight into the species involved in bacterial contamination of PCs and identify the source of bacterial contamination, CNS strains that were cultured from PCs with the BacT/ALERT system were identified to the species level with three different methods.

The CNS strains were identified by the sequencing of ~500 bp of the 16S rRNA gene. This gene was chosen as a large amount of published sequence data is readily available in public databases. Most of the strains were determined with this method. However, because of poor discriminative power due to the high sequence similarity between some species, for example a 464 bp fragment of *S. capitis* and *S. caprae* displayed only a single bp difference (Becker *et al.*, 2004), the *sodA* gene, which had a higher divergence than the 16S rRNA gene (Ghebremedhin *et al.*, 2008), was sequenced for confirmation. This confirmed the poor discriminative power of 16S rRNA gene sequencing for some species as four *S. saccharolyticus* strains were initially misidentified as *S. caprae* by 16S rRNA gene sequencing.

As a fast alternative for identification compared to sequencing, the CNS strains were also identified by MALDI-TOF

Table 2. Species identification of CNS by MALDI-TOF MS

Genus, identification of the CNS strain to the genus *Staphylococcus* with an identification confidence between 50 and 55 %; MM, mismatch – identification of the CNS strain to a wrong species with an identification confidence between 70 and 89.9 %; NM, no match with the spectra in the database – no identification. *S.*, *Staphylococcus*.

Strain as identified by sequencing	Identification confidence by MALDI-TOF MS/SARAMIS					Total
	≥99.9 %	90–99.9 %	Genus	MM	NM	
<i>S. epidermidis</i>	30	5				35
<i>S. capitis</i>	11	4*				15
<i>S. warneri</i>	6	4				10
<i>S. saccharolyticus</i>			6		4	10
<i>S. hominis</i>	6	1				7
<i>S. pasteurii</i>				2	2	4
<i>S. saprophyticus</i>		1				1
<i>S. lugdunensis</i>		1				1
Total	53	16	6	2	6	83

*One *S. capitis* strain was identified as *S. caprae*/*S. capitis* by MALDI-TOF MS.

Fig. 1. AFLP analysis of *S. capitis* species (based on *sodA* gene sequencing) found in PCs. Strains clustering with a similarity below 35 % were defined as different CNS species. The cut-off value is represented by a dotted line. The reference strains were LMD 89.175 (*S. capitis*) and ATCC 12228 (*S. epidermidis*).

MS. Recent studies have demonstrated that MALDI-TOF MS is a powerful tool for the identification of bacterial strains with low costs of consumables, easy interpretable results and a fast turnaround time (Bergeron *et al.*, 2011; Cherkaoui *et al.*, 2010; Dupont *et al.*, 2010; van Veen *et al.*, 2010). Overall identification of the CNS strains with the MALDI-TOF MS was good. However, the applicability of MALDI-TOF MS is dependent on the reference strains included in the database, for example; *S. saccharolyticus* could not be identified in this study as it was not included in the database. Other difficulties were recognized with the identification of *S. pasteuri*, a species that was present in the database, but was misidentified or not identified at all. Misidentification of *S. pasteuri* with MALDI-TOF MS has been demonstrated before (Bergeron *et al.*, 2011; Dupont *et al.*, 2010) and is probably associated with an absence of sufficient spectra from suitable reference strains in the mass spectral database.

Typing of the CNS strains with AFLP confirmed the results found with sequencing and MALDI-TOF, and moreover explained ambiguous results. *S. saccharolyticus* strains that were identified as *S. caprae* strains with 16S rRNA gene sequencing and as *S. saccharolyticus* strains with *sodA* gene sequencing clustered with a similarity between 35 and 90 % with the *S. saccharolyticus* reference strain, defining them as *S. saccharolyticus*, thereby confirming the *sodA* gene sequencing. Strain 08019 that shared 99.4 % identity with the 16S rRNA fragment and 95.6 % identity with the *sodA*

fragment of *S. capitis*, and was identified as a *S. capitis* or *S. caprae* with MALDI-TOF, clustered with a similarity of <35 % with the other *S. capitis* strains and the *S. caprae* reference strain, defining it as a different, likely novel, species. All different strains from the blood bank services in The Netherlands and Canada that belonged to one species clustered randomly. This demonstrates that within a species there is not a unique strain or species that is better adapted to survive in contaminated PCs.

Of all CNS species found from positive BacT/ALERT culture bottles, *S. epidermidis* was found predominantly, which is in agreement with results from other countries that screen PCs for bacterial contamination (Greco *et al.*, 2008; Jacobs *et al.*, 2008; Satake *et al.*, 2009; Walther-Wenke *et al.*, 2010; Zhu *et al.*, 2009). The other CNS species found corresponded to a large extent with the CNS species found in a study that determined the spectrum of bacteria detected in PCs in Germany (Walther-Wenke *et al.*, 2010). The CNS species found originate most likely from the normal skin flora (Gao *et al.*, 2007) with one remarkable exception; *S. pasteuri*. This bacterium is commonly isolated from naturally fermented Italian sausages, but has rarely been collected from human specimens. Recently *S. pasteuri* has been identified twice from contaminated PCs in Italy. Both contaminated PCs were detected before they were transfused (Savini *et al.*, 2008, 2009). In the first case it was found that *S. pasteuri* was cultured from swabs from the hands of the nurses who

Fig. 2. AFLP analysis of *S. epidermidis* strains (based on 16S rRNA gene sequencing) found in PCs. Strains clustering with a similarity between 80 and 100 % were defined as identical CNS strains. Strains clustering with a similarity above 35 % were defined as CNS strains of the same species and strains clustering with a similarity below 35 % were defined as different CNS species. The cut-off values are represented by dotted lines. The reference strains were ATCC 14990 and ATCC 12228 (both *S. epidermidis*), and LMD 89.175 (*S. capitis*).

were in charge of collecting the platelets, but it was not determined by typing if these strains were identical to the strain found in the PC (Savini *et al.*, 2008). *S. pasteurii* is not known as transient skin flora and adverse transfusion reactions caused by this bacterium are unknown. *S. saccharolyticus*, a strictly anaerobic resident of the skin flora (Evans *et al.*, 1978) was found relatively often at Sanquin but never at the CBS, where anaerobic cultures are not performed (Ramírez-Arcos *et al.*, 2007). This bacterium has never been implicated in transfusion reactions and is rarely encountered in patients, but can be of clinical significance (Wu *et al.*, 2009). The finding of this strict anaerobic species supports the importance of anaerobic culturing.

When a PC is found positive for bacterial contamination at Sanquin the PC, if available, is removed from the supply and a reculture of the product is started as soon as possible. A large amount of these positive PCs were negative on reculturing. According to the Canadian definitions, these would be classified as false positives due to contamination (Ramírez-Arcos *et al.*, 2007). However, in the Netherlands false positives are defined differently, only positively flagged bottles from which no micro-organism is recovered are called false positives. The reason for a negative result in the second culture might for example be, as has been shown for *S. epidermidis* (Brecher *et al.*, 2000), that bacteria in PCs can show slow and varied growth compared to the BacT/ALERT culture. Also, not all bacteria that are initially present in PCs proliferate; some bacteria have the property of being susceptible to self-sterilization or so-called auto-sterilization in PCs (Ezaki *et al.*, 2007). Moreover, due to the 'negative to date' release policy, a large percentage of PCs is not available for reculturing. Fifteen initial positive PCs cultures could not be confirmed by reculturing of the positive BacT/Alert bottle. Two of these were already transfused but neither was involved in a transfusion reaction nor the septic period (Koopman *et al.*, 2009).

Some *S. epidermidis* strains from the CBS collection showed a potential enhanced virulence due to the ability of these strains to produce biofilms (Greco *et al.*, 2008). Biofilms consist of slow-growing surface-attached bacterial cell aggregates embedded in a slimy matrix with increased antibiotic resistance that are able to evade the immune system and cause chronic infections (Götz, 2002). Earlier it was demonstrated that the storage conditions of PCs support the formation of biofilms on platelet aggregates and on PC bags (Greco *et al.*, 2007). Preliminary results showed that from the Dutch strains that were positive on reculture, one *S. capitis* strain was able to form biofilms (H. Ali, S. Uzicanin, M. Jacobs, R. Yomtovian, I. G. H. Rood, D. de Korte & S. Ramírez-Arcos, unpublished data).

The implementation of a diversion bag into which is removed the first 20 to 30 ml of the blood donation for pre-donation sampling, and improvement of skin disinfection, have proven to be effective in reducing the frequency of contaminated blood products, especially for contamination

with resident skin flora (de Korte *et al.*, 2006). However, the most frequently identified bacterial contaminants of PCs are still bacteria that are part of the normal skin bacteria. This suggests that there is a need for improved skin disinfection (Ramírez-Arcos & Goldman, 2010), which can easily be evaluated in the bacterial screening of PCs. However, some skin contaminants reside in the lower skin layers and no disinfection method would reach those layers, indicating the need for a different approach.

In conclusion, 83 CNS strains were analysed with three different methods; sequencing, MALDI-TOF MS and AFLP. All species could be identified by 16S rRNA or *sodA* sequencing, making this the most suitable method for the identification of CNS strains. Identification with MALDI-TOF MS looked promising. However, not all CNS species were present yet in the MALDI-TOF MS database. Eight different CNS species were identified with *S. epidermidis* being the predominant isolate. The species identified are mainly recognized as part of the normal skin flora. AFLP analysis showed that there is not a unique CNS bacterium that is involved in the contamination of PCs.

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