Characterization of the growth dynamics and biofilm formation of *Staphylococcus epidermidis* strains isolated from contaminated platelet units

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Bacterial contamination of platelet concentrates (PCs) poses the highest transfusion-associated infectious risk, with *Staphylococcus epidermidis* being a predominant contaminant. Herein, the growth dynamics of 20 *S. epidermidis* strains in PCs and regular media were characterized. Strains were categorized as fast (short lag phase) or slow (long lag phase) growers in PCs. All strains were evaluated for the presence of the biofilm-associated icaAD genes by PCR, their capability to produce extracellular polysaccharide (slime) on Congo red agar plates and their ability to form surface-attached aggregates (biofilms) in glucose-supplemented trypticase soy broth (TSBg) using a crystal violet staining assay. A subset of four strains (two slow growers and two fast growers) was further examined for the ability to form biofilms in TSBg. Two of these strains carried the icaAD genes, formed slime and produced biofilms in TSBg and PCs, while the other two strains, which did not carry icaAD, did not produce slime or form biofilms in TSBg. Although the two ica-negative slime-negative strains did not form biofilms in media, they displayed a biofilm-positive phenotype in PCs. Although all four strains formed biofilms in PCs, the two slow growers formed significantly more biofilms than the fast growers. Furthermore, growth experiments of the two ica-positive strains in plasma-conditioned platelet bags containing TSBg revealed that a slow grower isolate was more likely to escape culture-based screening than a fast grower strain. Therefore, this study provides novel evidence that links *S. epidermidis* biofilm formation with slow growth in PCs and suggests that slow-growing biofilm-positive *S. epidermidis* would be more likely to be missed with automate culture.

INTRODUCTION

In modern transfusion medicine, bacterial contamination of blood products represents the most prevalent transfusion-associated infectious risk (Corash, 2011). Platelet concentrates (PCs) are the most frequently contaminated blood product due to their storage conditions, which are amenable to bacterial growth (Corash, 2011). Implementation of several strategies has led to lower blood product contamination rates (de Korte et al., 2006). American centres using an improved donor skin disinfection method reported a significantly lower rate of PCs testing positive for bacterial contamination in comparison with control centres (100 vs 214 per million) (Benjamin et al., 2011). Similarly, introduction of first aliquot diversion resulted in a 47% reduction in contamination in the UK (McDonald, 2011). Platelet screening for bacterial contamination has prevented septic transfusion reactions by removal of contaminated PCs from inventories worldwide (Corash, 2011). Despite these mitigation measures, there are still sporadic reports of transfusion reactions due to contaminated PCs that escape detection during platelet screening (Benjamin et al., 2014). Some European countries have taken additional measures to reduce the risk of transfusing bacterially contaminated PCs. In Germany, PCs are retested on days 3, 4 and 5.
post-collection, since detectable levels of contamination are usually reached late during platelet storage (Sireis et al., 2011). European centres that have implemented pathogen-reduction technologies in the last decade have not reported platelet transfusion-related sepsis (Corash, 2011). While Gram-negative bacteria are typically implicated in the most severe instances of adverse transfusion reactions (ATRs) resulting from PC contamination, Gram-positive organisms are the bacteria most often recovered from contaminated platelet units (Corash, 2011; Ramirez-Arcos et al., 2007; Yomtovian et al., 2006).

Coagulase-negative staphylococci (CoNS) are Gram-positive commensal inhabitants of the human skin and comprise the most frequent platelet contaminants. These bacteria are likely introduced into PCs by venepuncture at the time of blood collection. Staphylococcus epidermidis is the most abundant CoNS and is the staphylococcal species most frequently isolated from contaminated PCs (Corash, 2011; Jacobs et al., 2008; Walther-Wenke et al., 2010). Instances of ATRs involving S. epidermidis have been reported worldwide and include several reactions with fatal outcomes (Ramirez-Arcos et al., 2007; Yomtovian et al., 2006; Jacobs et al., 2008; Walther-Wenke et al., 2010; Goldman et al., 2001; Hsueh et al., 2009; Martini et al., 2012). Although S. epidermidis is a ubiquitous component of the skin microflora, it is currently recognized as an important opportunistic pathogen, particularly in a nosocomial context, and is the leading cause of indwelling medical prosthestis infections (Otto, 2012; Costerton et al., 2009). To the authors’ knowledge, studies comparing characteristics of S. epidermidis strains isolated from PCs and biomedical devices have not been reported. However, it is expected that there would be more selective pressure for virulence and antimicrobial resistance on hospital isolates than on strains isolated from healthy blood donors.

The pathogenesis of S. epidermidis is largely related to its ability to form biofilms, defined as surface-associated bacterial aggregates encased within an extracellular polymeric matrix (Fey & Olson, 2010). Biofilms enable bacterial populations to resist mechanical stress, disinfection and antibiotic treatment, and evade the immune system. The S. epidermidis biofilm matrix comprises numerous bacterial substances, including proteins associated with adhesion and accumulation, teichoic acids, and exopolysaccharide. Perhaps the most well-characterized component of the S. epidermidis biofilm is the polysaccharide intracellular adhesin (PIA), a homopolymer of poly-N-acetylglucosamine, which constitutes the main component of the extracellular matrix (Mack et al., 1996). Biosynthesis and export of PIA are mediated by factors encoded in the icaADBC operon (Fey & Olson, 2010; Heilmann et al., 1996). Interestingly, numerous PIA-negative biofilm-forming strains of S. epidermidis have been isolated from clinical environments, demonstrating the numerous factors that influence biofilm formation under physiological conditions (Christner et al., 2010; Petrelli et al., 2006; Qin et al., 2007; Rohde et al., 2007).

Of interest to the present study are our previous observations that many strains of S. epidermidis and other CoNS isolated from contaminated PCs form biofilms under platelet storage conditions (Greco et al., 2007, 2008; Greco-Stewart et al., 2013). Remarkably, S. epidermidis PIA-negative strains, which fail to form biofilms in traditional laboratory medium, adopt a biofilm-positive phenotype in PCs (Greco et al., 2007). Potential clinical implications can be speculated since we have recently also shown that PIA-negative strains grown in PCs display increased pathogenicity in a nematode model (Hodgson et al., 2014). Furthermore, we have demonstrated that biofilm formation by the Gram-negative bacterium Serratia marcescens in PCs is associated with reduced bacterial detection by automated culture-based screening using the BacT/ALERT 3D culture system (bioMérieux) (Greco-Stewart et al., 2012). Bacterial aggregation and association with platelet storage bag materials likely reduce the availability of free-floating cells for sampling resulting in missed detection. Interestingly, the platelet storage environment also triggers biofilm formation by biofilm-negative Serratia marcescens strains (Greco-Stewart et al., 2012). To the authors’ knowledge, pathogen-reduction technologies have not been tested for their ability to reduce or eliminate bacterial biofilms in PCs. The present study sought to characterize strains of S. epidermidis recovered from contaminated platelet units from three different countries and spanning nearly two decades of surveillance. Growth characteristics of these strains and their capacity to form biofilms in PCs, using parameters such as PIA production and the presence of biofilm icaAD genes, were assessed. Finally, experiments were designed to determine whether adhesion to the plastic of platelet containers was associated with an increased propensity of S. epidermidis to avoid detection by the BacT/ALERT system when grown in regular laboratory media.

**METHODS**

**Bacterial strains and growth conditions.** A total of 20 S. epidermidis strains isolated from contaminated platelet units were characterized in the present study. A study conducted by Jacobs et al. (2008) showed that out of 54 contaminated platelet units evaluated in the United States from 1991 to 2006, 38 (70%) were contaminated with S. epidermidis. Strains used in this study were collected from the United States (strains 11-92, 02-96, 03-96, 04-96, 07-98-1, 07-98-2, 07-98-3, 07-98-4, 10-99, 07-04 and 09-04) (Yomtovian et al., 2006), Canada (strain 02-09) (Ramirez-Arcos et al., 2011) and The Netherlands (strains 1025504, 1025522, 1025524, 1025527, 1025679, 930892-4, 21073-5 and 22334-6) (Rood et al., 2011). While the American strains were isolated from contaminated PCs that had been transfused, and in some cases involved in ATRs, the Dutch strains were isolated from contaminated units that were detected prior to transfusion during routine platelet screening. The Canadian strain was isolated during rescreening of outdated PCs that had yielded negative results during initial screening (i.e. false-negative culture). Strains were preserved in brain heart infusion (BHI) broth (BD Biosciences) containing 15% glycerol, stored at ~80°C. S. epidermidis strains ATCC 35984 and ATCC 12228 were purchased from the American Type Culture Collection (Manassas, VA, USA) and used as biofilm-positive and -negative control strains, respectively. Strains were grown...
on tryptase soy agar (TSA; BD Biosciences) or in tryptase soy broth (TSB; BD Biosciences) and incubated aerobically overnight at 37 °C. TSB was supplemented with 0.5% glucose (Difco) (TSBg) for biofilm-formation assays.

Prior to commencement of this investigation, the identity of each isolate was confirmed using the Analytical Profile Index (API) Staph kit (bioMérieux), including catalase and lysozyme tests. Additional confirmation of identity was achieved through amplification of the cell division gene _divIVA_ using primer pair Staphdiv-FW (5'-GGC CGT CGA CAT GCC TTT TAC ACC AAG TG-3') and Sepdiv-REV (5'-GGC CCG ATC CTT AAT TAT TTG ATG TTG ATTG-3'); amplification of this gene has previously been shown to discriminate between _S. epidermidis_ and other CoNS species (Mastronardi & Ramirez-Arcos, 2007).

For experiments requiring a particular initial bacterial concentration, enumerated frozen stocks were prepared as previously described (Greco-Stewart et al., 2012). Briefly, bacterial pellets were resuspended in BHI + 15% (v/v) glycerol and adjusted to a McFarland turbidity standard of 0.5 corresponding to a concentration of ~10^8 c.f.u. ml^-1. The suspension was divided into 1 ml aliquots and preserved at ~80 °C. Serial dilutions of this preparation were concurrently plated on TSA and incubated aerobically overnight at 37 °C in duplicate for enumeration. When aliquots were thawed for experimentation serial dilutions were also plated and enumerated in duplicate as described to confirm each experimental inoculum.

**PCs.** Whole blood and apheresis PCs were collected from healthy volunteer donors with informed, signed consent, and screened for sterility and transmissible diseases. Outdated PCs were provided by Canadian Blood Services centres across Canada, whereas fresh (in-date) PCs were manufactured at the Canadian Blood Services Network Centre for Applied Development (netCAD; Vancouver, BC, Canada). Platelet collection, manufacture (buffy-coat and apheresis methods) and storage were performed in accordance with Canadian Blood Services Standard Operating Procedures. The use of PCs and research design were approved by the Canadian Blood Services Research Ethics Board.

**Growth of _S. epidermidis_ in media and PCs.** For growth curves in regular media, _S. epidermidis_ strains were streaked onto TSA plates and incubated aerobically overnight at 37 °C. Distinct colonies were selected and inoculated into 15 ml TSB, followed by overnight incubation at 37 °C with agitation at 260 r.p.m. The following day, a culture flask containing 100 ml TSB was inoculated with overnight culture to an OD_550_ of 0.05 (corresponding to approximately 1 x 10^7 c.f.u. ml^-1). The flask was incubated aerobically at 37 °C with agitation at 260 r.p.m. and 100 µl samples were taken every 2 h for a period of 10 h. The samples were serially diluted up to sevenfold when required, and the last three dilutions were plated on TSA plates in duplicate. The next day, colonies were counted and bacterial concentration was determined. Each growth curve was repeated when required, and the last three dilutions were plated on TSA plates in duplicate. The next day, colonies were counted and bacterial concentration was determined. Each growth curve was repeated when required, and the last three dilutions were plated on TSA plates in duplicate. The next day, colonies were counted and bacterial concentration was determined. Each growth curve was repeated when required, and the last three dilutions were plated on TSA plates in duplicate. The next day, colonies were counted and bacterial concentration was determined.

For growth curves in PCs, _S. epidermidis_ strains were grown overnight on TSA plates and incubated aerobically at 37 °C. Distinct colonies were selected and inoculated into 15 ml TSB, followed by overnight incubation at 37 °C with agitation at 260 r.p.m. Cell concentration was adjusted to ~100 c.f.u. ml^-1 in 100 ml outdated PCs, corresponding to approximately 1/3 of 1 platelet unit, in a vented polypropylene tissue culture flask (75 cm^2_, canted neck; BD Biosciences). Flasks were incubated under platelet storage conditions (22 ± 2 °C for 5 days with horizontal agitation at ~60 r.p.m.). Use of vented flasks for growth experiments allowed for more efficient use of PCs when testing the large number of bacterial strains used in this study. _S. epidermidis_ growth patterns in these flasks do not differ from those observed when growth experiments are performed in platelet units as reported previously by our laboratory (Greco et al., 2010) and by Dumont et al. (2011). Daily samples of 100 µl were recovered and serial dilutions were plated in duplicate for bacterial enumeration. Growth experiments were performed twice in duplicate for all strains, but at least three more independent replicates, each in duplicate, were performed for the group identified as slow growers in PCs to confirm this growth pattern.

**Detection of PIA using a Congo red agar (CRA) assay.** A CRA assay was performed based on the methods of Freeman et al. (1989) and adapted as previously reported (Greco et al., 2008). Congo red dye (Sigma-Aldrich) and sucrose (Sigma-Aldrich) were dissolved in sterile H_2O, filter-sterilized and added to autoclaved BHI agar to final concentrations of 0.8 and 36 g l^-1, respectively. Plates (~20 ml) were poured and stored at 4 °C. Strains of _S. epidermidis_ were streaked onto CRA, incubated overnight at 37 °C and scored as CRA-positive (black, crystalline morphology) or CRA-negative (white-to-pink, smooth morphology). Experiments were performed in duplicate.

**Amplification of _icaA_ and _icaD_.** _S. epidermidis_ ATCC 35984 (GenBank accession no. NC_002976.3) was used to design specific primers for _S. epidermidis_ icaA and icaD. The _icaA_ gene was amplified using primer pair Sepi_icaA-FW (5'-GGG CCG CCT GGA TAG TAG GAT CGA TTG AC-3') and Sepi_icaA-REV (5'-GGG CTT ACC GTT GGA TAT TGC CTC T3'), which anneal to nucleotides 51 to 72 and 1218 to 1239, respectively. For the amplification of _S. epidermidis_ icaD, primer pair SepicaD-FW (5'-AGG CCC AGA CAG AGG CAA TAT CCA-3') and SepicaD-REV (5'-AGT ACA AAG AAA CTC ATC CAT CCG A-3'), which anneal to nucleotides 7 to 30 and 215 to 239, respectively, were employed. Primers were synthesized by Integrated DNA Technologies. Reactions were performed using HotStar Taq Plus DNA polymerase (Qiagen) with an annealing temperature of 58 °C and an extension time of 30 s. Bacterial cell suspensions adjusted to 0.5 McFarland turbidity standard were used as templates. icaD-positive (ATCC 35984) and -negative (ATCC 12228) _S. epidermidis_ strains were included as positive and negative controls, respectively.

**Biofilm-formation assay.** Semiquantitative biofilm-formation assay by crystal violet (CV) staining was performed as previously described (Greco et al., 2007, 2008) based on the methods of Christensen et al. (1982), and using ATCC 35984 and ATCC 12228 as biofilm-positive and -negative controls, respectively. _S. epidermidis_ strains were grown in TSBg and incubated aerobically overnight at 37 °C with agitation (~260 r.p.m.). TSB was supplemented with glucose since it has been demonstrated that it is a requirement for PIA-dependent biofilm formation (Greco et al., 2007; Christensen et al., 1982). Cultures were diluted to OD_600_ 0.1 in 10 ml TSBg and 3 ml aliquots were transferred into the wells of a six-well tissue-culture-treated polystyrene plate (Fisher Scientific) and incubated at 37 °C overnight without agitation. Wells were aspirated of planktonic cells and media, rinsed three times with sterile PBS (pH 7.4), and stained with 2 ml 0.1% Gram CV (BD Biosciences) for 30 min at room temperature, followed by agitation (~100 r.p.m.). The dye was then removed, wells were rinsed three more times with PBS and biofilms were eluted in 3 ml of 80% ethanol:20% acetone (v/v) for 15 min with agitation (~100 r.p.m.). Eluate was transferred in triplicate to a 96-well microtitre plate (Fisher Scientific; 200 µl per well) and the absorbance was measured at a 492 nm wavelength in a microplate reader (Expert Plus).

Previous research in our laboratory has demonstrated that biofilm-formation studies of _S. epidermidis_ performed in outdated PCs lack reproducibility (Greco et al., 2007) and therefore biofilm-formation assays should be conducted in fresh (in-date) PCs. Due to the limited availability of fresh PCs, only a subset of _S. epidermidis_ strains...
(02-96, 07-04, 02-09 and 09-04) was chosen for further analysis of their biofilm-associated characteristics. For these experiments, 10 ml aliquots of fresh platelets were inoculated with overnight cultures to a final concentration of OD\textsubscript{600} 0.1 and dispensed into six-well plates as above. Plates were incubated under platelet storage conditions prior to CV staining. Assays were performed in triplicate in two separate experiments.

**Missed bacterial detection experiments.** We have recently demonstrated that adhesion, the initial step of biofilm formation, contributes to missed detection of the Gram-negative bacterium *Serratia marcescens* in PCs when screened with the BacT/ALERT system (Greco-Stewart et al., 2012). The experiments described herein followed a similar approach (Fig. 1); they were designed to determine if adhesion of *S. epidermidis* to the inner surface of platelet containers contributes to failed detection by the BacT/ALERT system. Following initial characterization, two biofilm-positive strains were selected for these experiments, including the slow grower *S. epidermidis* 02-96 and the fast grower strain 07-04. Both strains are clinical isolates from the United States that caused ATRs upon transfusion (Yomtovian et al., 2006). Plasma-conditioned platelet storage bags were obtained by evacuating the contents (outdated PCs) followed by filling with 200 ml TSBg. The experiments were performed in TSBg due to lack of availability of fresh PCs. Bags were then inoculated with *S. epidermidis* 02-96 and *S. epidermidis* 07-04 to final concentrations of approximately 0.1 c.f.u. ml\textsuperscript{−1} (20 c.f.u. per bag) and 0.3 c.f.u. ml\textsuperscript{−1} (60 c.f.u. per bag) using enumerated frozen stocks. These concentrations were selected to approximate the levels of contamination that are predicted to occur in a clinical setting (Benjamin & Wagner, 2007). Following inoculation, bags were incubated at room temperature with gentle agitation (~60 r.p.m.) for 2 h after. After the 2 h incubation, samples of 8–10 ml PCs from each bag were withdrawn to inoculate each of 10 blood products aerobic (BPA) culture bottles (bioMérieux). Bottles were incubated in the BacT/ALERT system until positive or for up to 6 days, and the number of positive bottles was documented. A 2 h incubation was chosen since *S. epidermidis* is a slow grower and this is the estimated time *S. epidermidis* takes to initiate biofilm formation according to preliminary assays performed in our laboratory. Our previous work has shown that fast-growing bacteria such as *Serratia marcescens* take only 20 min for adhesion to platelet bags (Greco-Stewart et al., 2012). These experiments were repeated five and three times for the 0.1 and 0.3 c.f.u. ml\textsuperscript{−1} inocula, respectively.

**Statistical analyses.** Mean and SD of the growth rate and biofilm formation were calculated using Excel 2003 (Microsoft). To compare biofilm formation between slow- and fast-growing strains in PCs, mixed models with random effects were fitted to perform comparisons between the biofilm-negative strain *S. epidermidis* ATCC 12228 and each of the other strains. Dunnett adjustment was applied to correct the larger false-positive error due to multiple comparisons. Differences in growth during the missed detection experiments were calculated by pairwise comparison using the Statistical Analysis System SAS 9.1.3 software (SAS Institute). A value of \( P < 0.05 \) was interpreted as significant.

**RESULTS**

*S. epidermidis* strains display different growth patterns in PCs

All strains were confirmed to be *S. epidermidis* as per API identification and the presence of the *divIVA* gene. The 20 strains tested in this study grew in TSB reaching concentrations of \(10^{10}\) to \(10^{12}\) c.f.u. ml\textsuperscript{−1} after 10 h incubation. Major
differences in the growth patterns between isolates were not observed in TSB with generation times ranging from 0.84 h for strain 07/04 to 0.67 h for strain 02/09. Although all strains were able to proliferate in PCs under platelet storage conditions, marked differences in their growth rate were detected (Fig. 2, Table 1). By the end of day five of incubation in PCs, it was apparent that two different patterns of growth had emerged: fast growers (15 strains, Fig. 2), characterized by a short lag phase (1–2 days) and high final bacterial concentration ($10^9$ c.f.u. ml$^{-1}$), and slow growers (five strains, Fig. 2) that had a longer lag phase (2–3 days) and lower final bacterial concentration ($10^3$–$10^6$ c.f.u. ml$^{-1}$). The generation times for the strains grown in PCs ranged from 13.44 h (strain 1025679) to 55.44 h (strain 02-96) in the slow-growing group, and from 6.48 h (strain 07-98-3) to 11.79 h (strain 21073-5) in the fast-growing group.

Presence of biofilm-associated genes and slime formation is predominant in S. epidermidis strains able to produce biofilms in regular media

As illustrated in Table 1, 4 out of the 20 S. epidermidis studied herein carried the biofilm-associated icaAD genes, produced slime on CRA and had a biofilm-positive phenotype when grown in TSBg. One strain, S. epidermidis 07-98-4, failed to produce biofilms in TSBg despite production of slime and the presence of the ica genes. The remaining 15 strains had a biofilm-negative phenotype supported by the absence of the ica genes and failed slime production on CRA.

Slow growth of S. epidermidis in PCs is associated with a greater amount of biofilms

A subset of four S. epidermidis strains, two fast growers and two slow growers, were selected to test their ability for biofilm formation in PCs. S. epidermidis strains 02-96 and 07-04, slow- and fast-grower strains, respectively, both possess the icaAD genes, demonstrate a PIA-positive phenotype on CRA, and formed biofilms in both TSBg

### Table 1. Growth and biofilm-associated characteristics of S. epidermidis strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth in PCs</th>
<th>Presence of:</th>
<th>Slime</th>
<th>Biofilm</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>icaA</td>
<td>icaD</td>
<td>TSBg</td>
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<tr>
<td>02-96*</td>
<td>Slow</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>07-04*</td>
<td>Fast</td>
<td>+</td>
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<td>1025679</td>
<td>Slow</td>
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ND, Not determined.

*Strains associated with ATRs (Yomtovian et al., 2006).
and PCs (Table 1, Fig. 3). The two other isolates, 09-04 (slow-grower strain) and 02-09 (fast-grower strain), were negative for the presence of the icaAD genes, slime production and biofilm formation in TSBg (Table 1). However, the latter two strains displayed a biofilm-positive phenotype in PCs (Table 1, Fig. 3). A similar conversion from a biofilm-negative to a biofilm-positive phenotype was observed with the biofilm-negative control strain ATCC 12228 (Fig. 3). Interestingly, the amount of biofilm formed by the slow-grower isolates was significantly higher than the amount formed by the fast-grower strains ($P<0.0001$).

**Slow-growing, biofilm-forming strains of S. epidermidis are more likely to escape detection by automated culture when the initial inoculum is low**

To examine the potential for biofilm-forming strains of *S. epidermidis* to escape detection by automated BacT/ALERT culture, an assay similar to that employed by Greco-Stewart *et al.* (2012) was designed (Fig. 1). Cultures of the strains 02/96 and 07/04, which both display biofilm-positive phenotypes in TSBg and in PCs (Table 1, Fig. 3), were chosen as representative strains for this experiment. Both strains were isolated from contaminated PCs that had been transfused resulting in ATR prior to the implementation of automated culture screening (Yomtovian *et al.*, 2006). In this experiment, platelet bags containing TSBg were inoculated at two initial concentrations, ~0.1 and ~0.3 c.f.u. ml$^{-1}$, since these values represent the typical low level of bacterial contamination found in a clinical setting (Benjamin & Wagner, 2007).

The slow-grower strain 02-96 was detected in 60% (30/50) and in 96.7% (29/30) of the bottles inoculated with ~0.1 and ~0.3 c.f.u. ml$^{-1}$, respectively, while the fast-grower strain 07-04 was detected in 74% (37/50) and 93.3% (28/30) of the bottles inoculated with ~0.1 and ~0.3 c.f.u. ml$^{-1}$, respectively. Pairwise comparison showed a significant difference in detection between the two inoculated concentrations for both strains ($P \leq 0.0005$). In particular, and most important, there was a significant difference in the number of positive bottles detected between the two strains at the 0.1 c.f.u. ml$^{-1}$ concentration of initial inocula ($P=0.0036$). The number of bottles detected with initial bacterial concentrations of ~0.3 c.f.u. ml$^{-1}$ was not significantly different between the two strains ($P=0.4439$).

**DISCUSSION**

In the present study, we have provided novel evidence that links slow growth with biofilm formation of *S. epidermidis* in the PC environment. It is interesting to note that different profiles of biofilm-associated properties are visible among strains. We have provided examples of both fast- and slow-growing strains that are able to form biofilms in TSBg, as well as those that form biofilms only in PCs. Examples of both fast- and slow-growing strains that possess icaAD were found and, in the present study, icaAD possession was correlated with a PIA-positive phenotype and production of biofilms in TSBg in the majority of the strains. The combinatorial approach of examining genetic as well as phenotypic aspects of these strains was used since possession of ica genes is not always correlated with a PIA-producing/biofilm-forming phenotype (Christner *et al.*, 2010; Petrelli *et al.*, 2006; Qin *et al.*, 2007; Rohde *et al.*, 2007). Together, these observations suggest the variability of characteristics of clinical *S. epidermidis* isolates, but reveal an underlying uniformity in their ability to convert to a biofilm-positive phenotype when grown in the PC environment in an ica-independent manner. It seems that conversion from a biofilm-negative to a biofilm-positive phenotype is common within CoNS since we recently described similar findings for another common platelet

![Biofilm formation of select S. epidermidis strains in TSBg and PCs.](http://jmm.sgmjournals.org)
contaminant Staphylococcus capitis (Greco-Stewart et al., 2013). Although the specific platelet storage factors that trigger biofilm formation by these organisms are so far unknown, the presence of platelets in plasma is a requirement for bacterial biofilm formation by S. epidermidis (Greco et al., 2007, 2010). Similar observations have been reported for streptococci (Jung et al., 2012), likely because platelets act as a scaffold for bacterial biofilm formation.

Of paramount importance to our studies was the establishment of the link between slow growth and biofilm formation of S. epidermidis in PCs, since fast growers produced significantly less biofilm than the slow growers. This observation could be interpreted to imply that slow-growing strains are not slow-growing per se, but rather have more growth associated with the biofilm rather than as suspended, planktonic (free-floating) cells. Bacteria encased in a biofilm matrix and attached to the walls of platelet containers would not be available for sampling. These data are further supported by our observation that S. epidermidis 02-96, a slow grower/heavy-biofilm-forming strain, was more likely to be missed by the BacT/ALERT system. Previous studies performed in PCs have reported that the BacT/ALERT system is effective in detecting many bacterial species at initial concentrations as low as 10 to 100 c.f.u. ml\(^{-1}\) (Brecher et al., 2001, 2005). However, S. epidermidis is estimated to contaminate PCs with initial inocula as low as 0.02 c.f.u. ml\(^{-1}\), and it is predicted that instances of false-negative screening will occur 70% of the time at this concentration (Benjamin & Wagner, 2007). Our analyses in TSBg demonstrated that levels of S. epidermidis of 0.1 c.f.u. ml\(^{-1}\) do escape detection, particularly when a heavy-biofilm-forming strain is the contaminating micro-organism. While it is expected that detection will not be complete at 0.1 c.f.u. ml\(^{-1}\) due to sampling error, the strain that formed more biofilms was reproducibly detected less often than its fast-growing counterpart. Data obtained from this study could be used as a basis to conduct similar assays in PCs to determine whether a relationship between biofilm formation and missed detection can also be shown for S. epidermidis in this milieu, as recently demonstrated for the Gram-negative bacterium Serratia marcescens (Greco-Stewart et al., 2012).

By improving methods of bacterial screening to account for reduced availability of biofilm-associated cells, it might become possible to capture more instances of contamination prior to transfusion, avoiding potential morbidity and mortality among transfusion recipients. It would be intriguing to know whether pathogen-reduction technologies would be able to eradicate bacterial biofilms formed during PC storage, which could be the subject of future studies.

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