

Revised Abstract

Background: Saphenous vein allografts are most commonly used for limb salvage procedures. Our laboratory evaluated bioburden levels before and after the processing and decontamination of saphenous vein grafts. We evaluated 918 saphenous veins donated between 2007 - 2010 to determine incoming bioburden levels, as well as the efficacy of the decontamination procedure. **Methods:** Veins are cultured for aerobic and anaerobic bacteria, and fungus upon receipt (pre-culture), and again after decontamination with an antimicrobial solution and preparation for cryopreservation (post-culture). **Results:** 638 (69%) of the pre-cultures yielded no growth, while 280 (31%) were positive, with 72 of the positive cultures (7.8%) yielding more than one organism. 889 (96.8%) of the post-cultures yielded no growth. Organisms isolated are listed in the following Table.

Organisms isolated from saphenous vein graft cultures.

Culture Result	No. pre-cultures	No. post-cultures
Negative	638	889
Mixed culture	72	16
CNS*	132	5
Bacillus sp.	13	0
Gram -negative bacilli	15	2
Anaerobes	21	6
<i>Staphylococcus aureus</i>	8	0
<i>Enterococcus faecalis</i>	2	0
Viridans streptococci	3	0
<i>Streptococcus pyogenes</i>	4	0
Diphtheroids	7	0

*Coagulase -negative staphylococci

Bioburden levels ranged from <100 colony forming units (cfu) /ml to >100,000 cfu/ml. Of the positive pre-cultures, 270 (96.4%) yielded <100 cfu/ml, while 10 (3.6%) grew >100 cfu/ml. Of the 29 positive post-cultures, 16 (55.2%) had mixed pre-cultures.

Conclusions: Evaluation of pre- and post-processing bioburden levels of saphenous vein allografts are important in trending recovery techniques, environmental conditions, culture collection and transport devices. Culture results are also necessary to determine the eligibility of a tissue for transplant. With over one million tissue transplants taking place each year, assessment of tissue quality and safety is essential.

Introduction

The prevalence of tissue allograft-associated infection is very low, but exact numbers are unknown. Reporting of such infections has been incomplete due to the lack of a national surveillance system. Non-viable allografts, such as bone, cartilage and tendons can be disinfected and sterilized prior to placement. While all tissue is rigorously screened and tested prior to transplant, grafts containing viable cells, such as heart valves, veins and corneas, can be treated with antimicrobials, but cannot be sterilized. Our laboratory cultured donated saphenous veins after initial processing, and again after antimicrobial treatment. This study evaluates the incoming bioburden and type of bacteria isolated prior to decontamination of 918 veins from 2007 to 2010.

Materials & Methods

Tissue is shipped from recovery agencies to our facility in an isotonic solution for surgical processing of the vein. Anaerobic cultures are collected prior to processing by generously swabbing the tissue with a swab and immediately inoculating the anaerobic transport device (Starswab Transport System, Starplex, Ontario, Canada). Prior to further decontamination procedures, aerobic, anaerobic and fungus cultures are set up. **Aerobic pre-treatment culture** – a SBAP is inoculated with 250 µl of isotonic solution from the specimen cup. The plate is streaked by making a series of passes through the inoculum in three planes. The culture is incubated in a CO₂ incubator at 35°C for 48 hours. **Fungus pre-treatment culture** – a Bactiflask containing Sabouraud-Dextrose agar with chloramphenicol (Remel, Lenexa, KS) is inoculated with 250 µl of isotonic solution from the specimen cup. The culture is incubated in a non-CO₂ incubator at 30° for 28 days. **Anaerobic pre-treatment culture** - an anaerobically reduced SBAP and an aerobic SBAP are inoculated with the swab from the Starplex transport in the anaerobic chamber. The reduced SBAP is incubated in the anaerobic chamber for seven days. The aerobic SBAP is incubated at 35°C in the CO₂ incubator for 48 hours. **Aerobic and anaerobic post-treatment cultures** – an Oxoid Blood Culture bottle previously inoculated by the vein processing technologist is incubated at 35°C for 7 days. **Fungus post-treatment culture** – a Bactiflask containing Sabouraud-Dextrose agar with chloramphenicol is inoculated by processing technologist. The culture is incubated in a non-CO₂ incubator at 30° for 28 days.

Results

Figure 1. Results of 918 saphenous vein cultures.

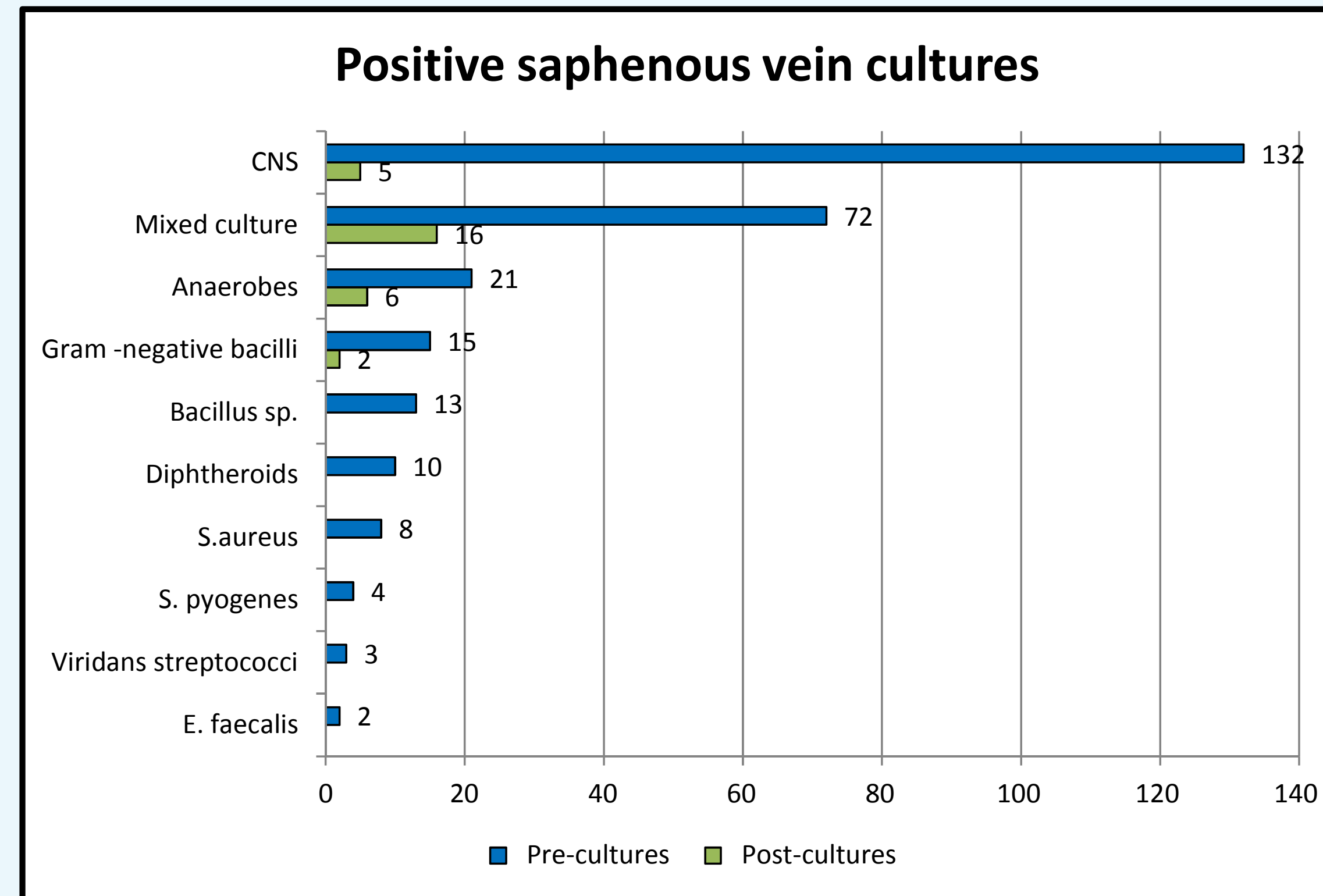


Table 1. Gram-negative bacilli isolated from 15 saphenous vein pre-treatment cultures.

Organism	Number of cultures positive
<i>Klebsiella pneumoniae</i>	2
<i>Enterobacter spp.</i>	3
<i>Escherichia coli</i>	4
<i>Acinetobacter spp.</i>	1
<i>Proteus mirabilis</i>	3
<i>Pseudomonas aeruginosa</i>	2

Table 2. Anaerobic bacteria isolated from 21 saphenous vein pre-treatment cultures.

Organism	Number of cultures positive
<i>Propionibacterium acnes</i>	16
<i>Clostridium perfringens</i>	2
<i>Clostridium sporogenes</i>	1
<i>Clostridium sordellii</i>	1
<i>Peptostreptococcus sp.</i>	1

Table 3. Semi-quantitative culture results from 309 positive saphenous vein cultures.

	Pre-culture	Post-culture
Positive (%)	280 (30.5)	29 (3.2)
Less than 100 cfu/ml (%)	270 (96.4)	27 (93.1)
Greater than 100 cfu/ml (%)	10 (3.6)	2 (6.9)

Discussion

Despite aseptic collection of donor veins, rigorous screening of transplant tissues is required to avoid transmission of infection. Retrospective studies may shed light on not just the prevalence of allograft-associated infections, but also the source of the bacterial contamination. Our results show the most common contaminant to be CNS, followed by mixed flora, anaerobes, and gram-negative bacilli. 96.4% of positive pre-cultures yielded <100 cfu/ml. Bacterial translocation from the GI tract does not seem to be a significant cause of contamination of saphenous veins, with only 18 (2.0%) cultures growing enteric bacilli. The fact that only a very small percentage (less than 1%) of veins were grossly contaminated with >100,000 cfu/ml indicates general adherence to aseptic recovery techniques. 69.5% of all pre-treatment cultures were negative, while 96.8% of post-treatment cultures were negative.

Conclusions

While overall contamination of saphenous allograft donor veins tested was 31%, most positive cultures (96%) yielded a bioburden of less than 100 cfu/ml, and 97% of all cultures were negative after decontamination treatment. All tissues positive on post-cultures are not eligible for transplant. Continued evaluation of incoming bioburden levels, and identification of the organisms will help monitor trends to determine the efficacy of the antibiotic decontamination process, as well as trending recovery techniques, environmental conditions, culture collection and transport devices. Above all, this information is necessary to determine the eligibility of a tissue for transplant. With over one million tissue transplants taking place each year, assessment of tissue quality and safety is essential.