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Anaerobic bioburden in transport solution of human cardiovascular tissues

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ABSTRACT

Although reports of infections caused by anaerobes after tissue transplantation are uncommon, contamination of allografts may result in substantial complications. Anaerobic incubation and testing of organ transport solution (TS) are not routine. The aim of this study was to determine the bioburden of strict anaerobic bacteria and oxygen tension of heart-TS. Forty TS from different donors were evaluated cultured using membrane filtration (MF), direct inoculation on broth and automated blood culture bottle (ABCB). Bacterial identification was performed by MALDI-TOF. The transport conditions were simulated to verify the bacterial recovery. A sterile bag fulfilled with 250 ml^{-1} of sterile saline was spiked with 100 CFU ml^{-1} of *Clostridium perfringens* and the fluid recovered 0 h, 1 h, 2 h, 6 h, 12 h, 24 h and 48 h for culture and oxygen measurement. *S*trict anaerobic bacteria were not isolated in heart-TS. The recovery of *C.perfringens* spiked in heart-TS was 100% using automated blood culture bottles. MF method detected > 100 CFU only after 6 h of spiking. The manual culture was not able to recover *C.perfringens* after the process. The percentage of O_2 measures varied from 77.6 to 87.9%. MF or ABCB are better than direct inoculation for recovery of anaerobes from heart-TS. Although all samples from heart donors were negative for anaerobes (probably due to low incidence of contamination), *C.perfringens* were all recovered in the simulated transport condition.

1. Introduction

Millions of tissue transplants are performed annually, and microbiological safety of the allograft is necessary for the quality of the transplantation. Although reports of infections after tissue transplantation are uncommon (Fishman et al., 2012), contamination of allografts may result in substantial complications or death (Kainer et al., 2004). In 2001, a fatal case of bacterial sepsis by *Clostridium sordelli* occurred in a 23-year-old man who received a cadaveric musculoskeletal allograft in the USA associated with contaminated allograft (Centers for Disease and Prevention, 2002). In allograft heart valve, cases of endocarditis have also been described (Agnihotri et al., 1995; Gall et al., 1995). Different tissues may experience different rates of contamination. Cardiovascular tissues contamination rate are usually higher than musculoskeletal tissue rates (Paolin et al., 2018). The contamination of cardiovascular allografts can occur in different steps, such as donor hidden infection, contamination from the environment, from contaminated supplies and reagents used during processing (Kowalski et al., 2012a, 2012b).

Methods to reduce bioburden have been used in tissue allografts to maximize microbiological safety for recipients of tissue allografts. Control measures are necessary to identify and eliminate contaminating microbes, monitor bioburden reduction by microbial testing at critical points, prevent further contamination and produce a finished allograft free from infectious organisms (AATB, 2016). Approximately 2.7% of retrieved heart valves showed some strict anaerobic bacterial growth in heart transport solution (heart-TS) (Sawa et al., 2019). Because of their fastidious nature, they are difficult to isolate and requires proper methods of collection, transportation and cultivation to maximize recovery.

During the recovery of the heart there may be contamination caused by donor microbiota, from the recovery staff during tissue recovery or environmental microorganisms (Schroeter et al., 2012). Therefore, it is important to perform microbiological examination of heart-TS that may reveal the presence of microorganisms. Specimens for anaerobic incubation and testing should be subjected to culture immediately after collection and subsequently incubated in a suited anaerobic environment. However, in a tissue bank temporary storage and transportation are unavoidable (Demuyser et al., 2018; Peterson, 1997). Problems relating to inadvertently exposing the bacteria to oxygen can arise at

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the time of specimen collection, during transport, and during the culture process (Peterson, 1997). Therefore, limited recovery can be due to problems in several key steps of the pre-analytical phase. There are no studies directly comparing the oxygen concentration in heart-TS and recovery of anaerobic bacteria mainly the non-spore-forming anaerobic bacteria.

The aim of this study was to determine the bioburden of strict anaerobic bacteria of heart-TS obtained from heart-beating and non-heart-beating donors using membrane filtration methodology and the direct incubation. We also evaluated the recovery potential of strict anaerobic bacteria in the presence of oxygen simulating the solution, temperature and different transport time.

2. Methods

2.1. Heart retrieval and transport

A total of 40 samples from different donors were evaluated from the Human Tissue Bank of Pontificia Universidade Católica do Paraná (HTB). After heart retrieval, the organ was placed in a tub with saline solution at 2–8 °C. The organ was placed in a sterilized plastic bag, immersed in ice-cold isotonic solution (heart-TS). This first packaging was sealed and then packaged in two more plastic bags. The triple package containing the organ was placed in an airtight container and placed inside a thermal box filled with ice and transported to the HTB.

2.2. Donor and heart transport characteristics

Information such as age, gender, type of death, city, hospital, use of antibiotics were obtained from medical records. Warm (WIT) and cold ischemic times (CIT) were also evaluated. The warm ischemia time is the time of cross clamping (or of asystole in non-heart-beating donors), until immersion in heart-TS. The cold ischemia time is the time that the heart was transferred to heart-TS until the time that the dissection of the valves was completed.

2.3. Visual classification of heart-TS

Firstly, the heart-TS total volume was evaluated using graduated glass measuring cylinder and the heart-TS visual appearance was classified according to its hemorrhagic aspect: none, mild, moderate or severe as previously described (Kraft et al., 2018).

2.4. Culture of heart-TS

The bioburden in heart TS was determined using membrane filter (MF) technique. One hundred milliliters of TS were filtered using a 0.45 μm sterile membrane filter systems (Millipore™, Cork, Ireland) simulating the tissue bank routine. The MF was placed onto Anaerinsol-S agar (Probac do Brasil, São Paulo, Brasil), a specific culture media for anaerobes with aminoglycoside (amikacin) and incubated for up to 72 h at 35 ± 1 °C in anaerobic atmosphere. Concomitantly, manual cultures using 90 ml^{-1} Thioglycolate (Laborclin, Pinhais, Brazil) were made using 10 ml^{-1} of the TS, which was obtained aseptically in a Class II-A laminar airflow cabinet with a syringe, equally distributed to the culture bottles and incubated up to 15 days, at 35 °C ± 1 °C. These cultures were examined daily for visual evidence of turbidity. For bacterial identification, isolates were identified using Matrix Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) (Bruker Daltonics, Bremen, Germany) according to the manufacturer's specifications, which is considered the gold standard for bacterial identification using the library (Biotyper database db6903; (V6 database))(van Belkum et al., 2017)

2.5. Anaerobic suitability of culture media

To confirm the media suitability, growth promotion test was performed with ≤100 colony-forming units of *Clostridium perfringens* (previously recovered from a clinical sample and identified by MALDI-TOF) onto 90 ml^{-1} Thioglycolate Broth. The media spiked with *C. perfringens* was incubated at 36.5 °C for 7 days in accordance to US Pharmacopoeia (USP, 2017). Negative controls were performed with Thioglycolate broth without samples to confirm broth sterility. After the incubation period, the growth promotion was considered suitable if clearly visible growth of microorganisms was seen, while the broth without microorganism should remain clear and without growth indicative (Fig. 2).

2.6. Testing of anaerobic viability of TS

The transport conditions were simulated to verify the recovery of anaerobic bacteria. In order to simulate a sample of infected heart-TS, a sterile bag fulfilled with 250 ml^{-1} of sterile saline (JP, Ribeirão Preto, Brazil) was spiked with an inoculum of 10^2 CFU ml^{-1} of *C. perfringens* made with 1:10 dilution from 0.5 McFarland standard using a turbidimeter (Alfakit, Florianópolis, Brazil) under aseptic conditions. The mixture in the bag was homogenized. The survival of the organisms was determined at 0 h, 1 h, 2 h, 6 h, 12 h, 24 h and 48 h. For each time, three bags were used, two for oxygen measurement with an oximeter (NASCO, Fort Atkinson, USA) and the other one for microbiological culture (duplicate).

One hundred milliliter of the suspension were filtered into a 0.45 μm membrane and placed onto Anaerinsol-S agar, which was incubated for 72 h under anaerobic conditions and evaluated. Moreover, conventional culture was performed using Thioglycolate broth (Laborclin, Pinhais, Brazil) for the same periods and incubated for up to 15 days. Concomitantly, automated cultures were also performed in the different times using Anaerobic bottles of BACTIME BC32 (Laborclin, Pinhais, Brazil) according to the manufacture's recommendations.

2.7. Statistical analysis

Continuous variables were expressed as mean with standard deviation (± SD) or median and interquartile range, while categorical variables were expressed as frequencies or percentages. This was a descriptive study and statistical calculi of comparison were used.

3. Results

3.1. Donor and heart transport characteristics

A total of 40 heart-TS was analyzed. The average age mean was 43.7 ± 12.8 years, 35% were from female and 65% were from male donors. 60% of the donors were from Paraná state, while 32.5% were from Bahia state, 5% were from Rio Grande do Norte state and 2.5% were from Ceará state. The mean WIT was 85.08 ± 108.95 min and the mean CIT was 1450.64 ± 803.50 min. Moreover, 30% of the donors were using antibiotics, among them: cefepime, ceftazidime, ceftriaxone + piperacillin/tazobactam, levofloxacin, or amikacin.

3.2. Visual classification of heart-TS

Considering the heart-TS visual hemorrhagic aspect (none, mild, moderate or severe), most of them were severe (62.5%) (Table 1 and Fig. 1). In 27.5% of heart-TS, residual blood with debris was present. Due to these conditions, filtration was not feasible to the entire volume. The mean volume used in each MF was 56.66 ± 33.12 ml^{-1}.

Table 1
Donor and heart transport-related factors.

Id	Age (Years)	Gender	City	State	WIT (Min)	CIT (Min)	TS hemorrhagic	TS volume (ml^{-1})
1	50	M	Curitiba	PR	0	570	+ + +	150
2	43	F	Maringá	PR	38	348	+	375
3	22	F	Vitória da Conquista	BA	20	2690	+ + +	250
4	52	M	Curitiba	PR	90	500	+ + +	100
5	33	M	Toledo	PR	14	1350	+ +	300
6	59	M	Vitória da Conquista	BA	35	1455	+ + +	250
7	42	F	Paranavaí	PR	35		+	350
8	41	F	Curitiba	PR	92	528	+ + +	175
9	59	M	Curitiba	PR	285	620	+ + +	140
10	52	M	Maringá	PR	23	1257	+	300
11	31	M	Curitiba	PR	110	974	+ +	150
12	44	M	Vitória da Conquista	BA	25	2875	+ + +	250
13	51	F	Campina Grande do Sul	PR	90	494	+ + +	200
14	60	M	Ponta Grossa	PR	35	407	+ +	325
15	56	F	Ponta Grossa	PR	20	1638	+ +	300
16	50	M	Curitiba	PR	370	1195	+ + +	125
17	53	F	Curitiba	PR	95	717	+	90
18	35	M	Campo Mourão	PR	25	1445	+ + +	270
19	40	F	Londrina	PR	75	455	+	300
20	39	M	Bahia	BA	30	2440	+ + +	180
21	22	M	Bahia	BA	16	2820	+	175
22	52	F	Maringá	PR	50	1925	+ + +	300
23	43	M	Curitiba	PR	117	237	+ +	250
24	34	M	Salvador	BA	63	1937	+ + +	200
25	54	M	Campo Mourão	PR	*	*	+ + +	300
26	55	M	Itabuna	BA	45	2407	+ + +	270
27	52	F	Foz do Iguaçu	PR	*	*	+	350
28	54	F	Natal	RN	3	1505	+ +	300
29	20	M	Feira de Santana	BA	*	*	+ + +	125
30	18	M	Fortaleza	CE	*	*	+	325
31	52	F	Campo Largo	PR	347	*	+ + +	150
32	35	M	Natal	RN	8	1303	+ + +	200
33	41	F	Salvador	BA	55	1835	+ + +	200
34	16	F	Salvador	BA	65	2055	+ + +	200
35	51	M	Salvador	BA	44	2387	+ + +	300
36	53	M	Guarapuava	PR	30	1865	+ +	260
37	53	M	Umuarama	PR	44	1220	+ + +	280
38	32	M	Salvador	BA	471	1103	+ + +	250
39	17	M	Foz do Iguaçu	PR	125	2695	+ + +	250
40	52	M	Salvador	BA	73	2070	+ + +	170

Legend: WIT = Warm ischemia time; CIT = Cold ischemia time; TS = transport solution; TS hemorrhagic; PR = Paraná; BA = Bahia; RN = Rio Grande do Norte; CE = Ceará. Classification = 0 none, + mild, + + moderate, + + + severe; * = no data available.

3.3. Strict anaerobic bacterial isolation and identification

In our study, 40 heart-TS samples were analyzed by qualitative microbiological culture and membrane filter technique. To allow growth of strict anaerobes on solid media, anaerobic jars were used together with gas generating, which release both CO_2 and H_2.

From 40 heart-TS samples inoculated directly in thioglycolate, no strict anaerobic bacteria were isolated. By MF methodology, only two heart-TS samples showed growth. The identification performed by MALDI-TOF classified the microorganisms as *Streptococcus anginosus* and *Staphylococcus capitis*, however these microorganisms are not strict anaerobic bacteria.

3.4. Anaerobic suitability of culture media

Thioglycolate broth showed growth promotion when positive control was analyzed, demonstrating that this media is suitable for *C. perfringens*. In addition, negative control did not show any growth and

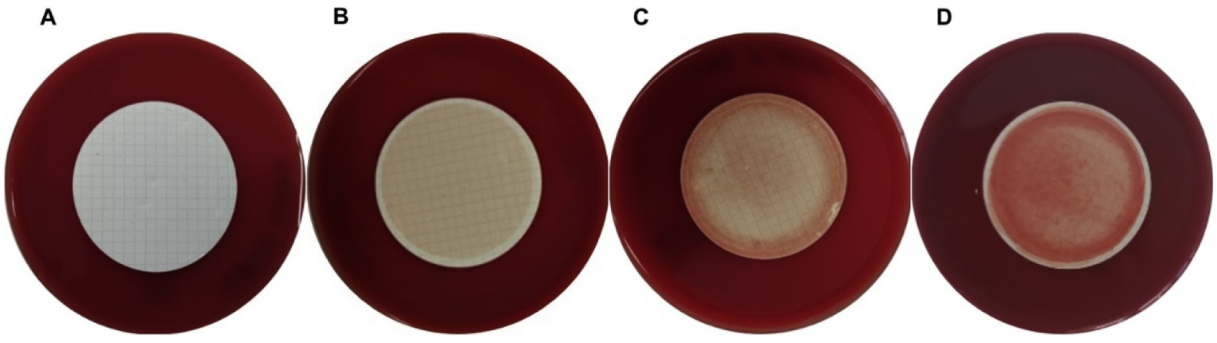

Fig. 1. Heart-TS visual hemorrhagic aspect, which was classified according to Kraft et al. 2018 as none, mild, moderate or severe. The classification was applied in the 40 routine samples as (A) 0 none, (B) + mild, (C) + + moderate and (D) + + + severe, where 62,5% of the samples were classified as severe.

Table 2
Simulated transport conditions and recovery rates.

Time	Oximeter			Oximeter			Membrane			Automated culture	Conventional culture
	%	mg/L	temp.	%	mg/L	temp.	CFU/ml^{-1}	CFU/ml^{-1}	Mean	Grown	Grown
0 h	82.1	6.9	23	80.3	6.8	23	0	0	0	+	–
1 h	77.6	6.6	18	83.1	6.9	18	0	2	1	+	–
2 h	82.7	6.7	10	78.1	6.6	10	0	6	3	+	–
6 h	81.3	6.6	10	80.8	6.7	10	> 100	> 100	> 100	+	–
12 h	87.9	8.6	10.4	83.1	8.9	10.4	> 100	> 100	> 100	+	–
24 h	84.4	8.9	11	83.9	8.8	11	> 100	> 100	> 100	+	–
48 h	85.2	9.3	9.4	86.4	9.6	9.4	> 100	> 100	> 100	+	–

turbidity, indicating that the media was not contaminated (sterile).

3.5. Survival of Clostridium perfringens after simulated transport

The recovery of *C. perfringens* stored in heart-TS is shown in Table 2. The results presented in Fig. 2 show that *C. perfringens* survives when stored in transport medium for up to 48 h. The membrane filtration method did not show any growth at time 0 h, while times 1 h and 2 h showed a mean of 1 and 3 CFU, respectively. From time 6 h to 48 h, growth was > 100 CFU, demonstrating that the *C. perfringens* was able to recover after a long period exposed to transport conditions. Moreover, the automated culture demonstrate growth in every hour, including time 0 h, demonstrating that this method could promote *C. perfringens* growth. However, the manual culture was not able to recover *C. perfringens* after the process.

The percentage of O_2 measures varied from 77.6 to 87.9% and mean of 82.6 ± 3.2%, while mg/L varied from 6.6 to 9.6 and mean of 7.7 ± 1.2 mg L^{-1}.

4. Discussion

This study evaluated strict anaerobic bacteria bioburden of heart-TS of donated human hearts. In addition, the effect of transport conditions on the survival of *C. perfringens* in heart-TS up to 48 h was evaluated. All TS were negative for strict anaerobes bacteria. The simulated TS spiked with *Clostridium* presented high levels of oxygen however, the bacteria were recovered in enriched media (automated culture).

Bioburden assessment testing must consider the type of organism and the numbers present. The method used must be able to recover a wide range of organisms that includes fastidious and non-fastidious organisms, spore-formers and non-spore formers. The method must also recover low numbers of organism that may be present. The methods for microbiological analysis vary widely among tissue banks and sampling occurs at critical points during heart valve processing. In HTB, the first microbiological culture is from heart-transport solution (TS). Heart-TS should be cultured in enrichment liquid cultures to maximize the recovery of aerobic and anaerobic bacteria and fungi. This is performed because the presence of spore-forming bacteria (*Clostridium* spp., *Bacillus* spp.), *Streptococcus pyogenes* and fungi/yeasts in heart-TS determines the rejection of the allografts from clinical use due to difficulties to eliminate these forms (Germain et al., 2016; Kainer et al., 2004).

In heart-TS, mixtures of several (both aerobic and anaerobic) species can be obtained (Heng et al., 2013; Kraft et al., 2018; Sawa et al., 2019). In our routine operations, heart-TS samples were used to a qualitative microbiological culture in thioglycolate, tryptic soy broth and Sabouraud dextrose for bacteriological and mycological testing. However, with this methodology, approximately 2.7% of retrieved heart valves showed some strict anaerobic bacterial growth in heart-TS (Sawa et al., 2019). Isolation rates of anaerobic bacteria from clinical material are highly variable. Some laboratories recover these organisms in as many as 25 to 50% of all specimens appropriate for anaerobic culture (Martin, 1971; Spaulding et al., 1972). Others isolate anaerobes only on rare occasions. Such divergent results have been often ascribed, in part, to differences in specimen transport. Exposure of clinical samples to oxygen is one of the main reasons for unsuccessful recovery of anaerobes. Although all isolates of anaerobes are not equally affected by the deleterious action of oxygen.

Bacterial contamination rates of heart-TS were reported in a recent work from our group. From 1.001 transport solution, 52% were contaminated. A total of 770 microorganisms were identified, and *Staphylococcus* spp. was identified in 248 isolates (32.2%). Skin bacteria from skin microbiota were the most commonly identified microorganisms (*Staphylococcus* spp., *Cutibacterium* spp., *Corynebacterium* spp., and *Bacillus* spp.), occurring in 49.6% (Sawa et al., 2019). However, the rate of tissue contamination varies between tissue banks. Paolin et al. found a contamination rate for cardiovascular tissues was 84% and more than one strain were simultaneously present in 44.6% of cardiovascular tissues (Paolin et al., 2017). Kraft et al. demonstrated that the bioburden of heart-TS in membrane filter is the technique of choice due to variation microorganisms detected (Kraft et al., 2018). In this study, was reported that from 20 samples in tryptic soy agar, 13 were positive (65%) with a mean count of 1.36 ± 4.04 CFUml^{-1}. Most isolates were Gram-positive cocci (10/20) and the second most frequent group was Gram-negative bacilli (4/20). Two fungi were identified in tryptic soy agar, being one yeast and one mold. However, the anaerobic microorganisms were not analyzed. According to Germain et al. the average bioburden of heart-TS obtained by membrane filter technique from 20 donors were 52 ± 212 CFUml^{-1} (range: 0–950) and 77 ± 245 CFUml^{-1} (range: 0–1040) for aerobic and anaerobic culture conditions, respectively (Germain et al., 2010). However, the bioburden has not been identified. Even though the application of different plates and incubation environments can selectively inhibit or stimulate the growth of certain species, careful assessment of the cultured specimen is always necessary (Demuyser et al., 2018).

However, none were positive for strict anaerobic bacteria. Based on these results, a model of artificial contamination of the heart-TS with *Clostridium perfringens* was performed. The recovery of *Clostridium perfringens* in the transport solution was analyzed by two methods, membrane filter technique and direct inoculation (manual and automated qualitative microbiological culture). However, the manual culture failed to detect the presence of *Clostridium perfringens*. This result was unexpected since the thioglycolate medium specifically selects for growth of anaerobic microorganisms. The MF technique is the gold standard, but this is a longer and a costly procedure (van Kats et al., 2010).

The membrane filter technique employed allows a quantitative measure of the inherent bioburden of heart-TS while direct inoculation allows a qualitative measure. However, using membrane filter technique may occur obstruction of the membrane filters and this is not a technique that is used routinely in most microbiological laboratories. Moreover, the growth of microorganisms can be inhibited by the presence of antibiotic residues retained in the membrane (AATB, 2016). We use anaerobic agar plates with addition of antibiotics

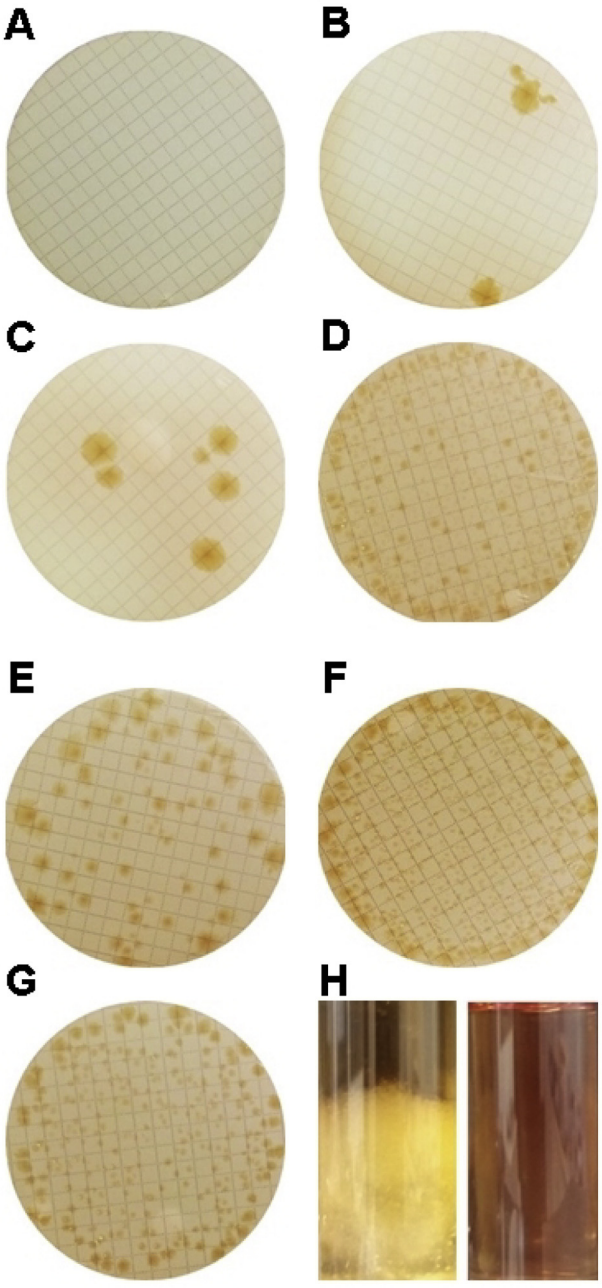

Fig. 2. Recovery of *Clostridium perfringens* by membrane filtration. *C. perfringens* survives when stored in transport medium for up to 48 h. The membrane filtration method did not show any growth at time 0 h, while times 1 h and 2 h showed a mean of 1 and 3 CFU, respectively. From time 6 h to 48 h, growth was > 100 CFU. (A) 0 h, (B) 1 h, (C) 2 h, (D) 6 h, (E) 12 h, (F) 24 h, (G) 48 h, (H) positive control (left) and negative control (right). Thioglycolate broth showed growth, demonstrating that this media is suitable for *C. perfringens*. Negative control did not show growth, indicating that the media was not contaminated.

(aminoglycoside - amikacin), which select only strict anaerobic microorganisms. These data support those observed in our study regarding many negative results. Moreover, the main problem for the cultivation of this kind of microorganisms is that they die, or immediately stop growing, upon exposure to low levels of oxygen. However, among anaerobic bacteria there is difference in the ability to survive in the presence of oxygen (Loesche, 1969; Tally et al., 1975). It has been proposed that differences in oxygen tolerance among anaerobes may be related to the effectiveness of defense mechanisms possessed by bacteria against toxic products of oxygen reduction (McCord et al., 1971). *Clostridium* spp. can form endospores allows them to survive in the presence of oxygen.

One of important contributor to microbial contamination of recovered tissue is bacteria translocation. Due to normal postmortem decomposition of the body occurs the movement of viable bacteria from the intestine to other body sites (Lichtman, 2001). Malinin et al. showed that 7% of cadaveric donors had *Clostridium* species in the bloodstream at the time of harvest (< 24 h after death) (Malinin et al., 2003). Presumably, dissemination to tissue occurs through the bloodstream because 4% of their patients had concomitant tissue cultures that were positive for the bacteria. Delayed removal of tissue from the cadaveric donor can possibly result in an increase of anaerobic and spore forming bacterial pathogens which are more resistant to disinfecting and sterilization procedures (Eastlund, 2006).

There are currently no guidelines or standard method for determining bioburden ssessment and microbiology laboratories may use different types of samples, culture media and methods. Culturing of strictly anaerobic bacteria in the absence of oxygen requires specific bacteriological techniques, which could explain the low frequency of isolation in many laboratories. Additional factors that may arise occasionally when the harvested tissue is delivered to a banking laboratory, such as the use of an adequate container, transport fluids or time and temperature limitations, can be related to the recovery of microorganisms.

5. Conclusions

The detection of strict anaerobic bacteria bioburden in samples of heart-TS was not possible with conventional culture, however, the conditions of the TS allow the growing of *Clostridium* in a model. Clinical validation using molecular methods (qPCR) inroutine would be helpful to improve these experimental findings.

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Ethical approval

This study was approved by the Ethics Committee of Pontificia Universidade Católica do Paraná (approval number 1.455.773).

Declaration of Competing Interest

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F. F. T. is CNPq researcher.

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References

AATB, 2016. Microbiological Process Validation & Surveillance Program, N° 5, Version 2, July 18, 2016 McLean, Virginia.
Agnihotri, A.K., McGiffin, D.C., Galbraith, A.J., O'Brien, M.F., 1995. The prevalence of infective endocarditis after aortic valve replacement. J. Thorac. Cardiovasc. Surg. 110, 1708–1720 (discussion 1720-1704).
Centers for Disease, C., Prevention, 2002. Update: allograft-associated bacterial infections–United States, 2002. MMWR. Morb. Mortal. Wkly Rep. 51, 207–210.
Demuyser, T., De Geyter, D., Van Dorpe, D., Vandoorslaer, K., Wybo, I., 2018. Extensive evaluation of fastidious anaerobic bacteria recovery from the Copan eSwab(R) transport system. J. Microbiol. Methods 144, 73–78.
Eastlund, T., 2006. Bacterial infection transmitted by human tissue allograft transplantation. Cell Tissue Bank. 7, 147–166.
Fishman, J.A., Greenwald, M.A., Grossi, P.A., 2012. Transmission of infection with human allografts: essential considerations in donor screening. Clin. Infect. Dis. 55, 720–727.
Gall, K., Smith, S., Willmette, C., Wong, M., O'Brien, M., 1995. Allograft heart valve

sterilization: a six-year in-depth analysis of a twenty-five-year experience with low-dose antibiotics. J. Thorac. Cardiovasc. Surg. 110, 680–687.
Germain, M., Thibault, L., Jacques, A., Tremblay, J., Bourgeois, R., 2010. Heart valve allograft decontamination with antibiotics: impact of the temperature of incubation on efficacy. Cell Tissue Bank. 11, 197–204.
Germain, M., Strong, D.M., Dowling, G., Mohr, J., Duong, A., Garibaldi, A., Simunovic, N., Ayeni, O.R., Bioburden Steering, C., Cardiac Tissue Working, g, 2016. Disinfection of human cardiac valve allografts in tissue banking: systematic review report. Cell Tissue Bank. 17, 593–601.
Heng, W.L., Seck, T., Tay, C.P., Chua, A., Song, C., Lim, C.H., Lim, Y.P., 2013. Homograft banking in Singapore: two years of cardiovascular tissue banking in Southeast Asia. Cell Tissue Bank. 14, 187–194.
Kainer, M.A., Linden, J.V., Whaley, D.N., Holmes, H.T., Jarvis, W.R., Jernigan, D.B., Archibald, L.K., 2004. Clostridium infections associated with musculoskeletal-tissue allografts. N. Engl. J. Med. 350, 2564–2571.
Kowalski, J.B., Merritt, K., Gocke, D., Osborne, J., 2012a. Assessment of bioburden on human and animal tissues: part 2–results of testing of human tissue and qualification of a composite sample for routine bioburden determination. Cell Tissue Bank. 13, 431–439.
Kowalski, J.B., Mosley, G.A., Merritt, K., Osborne, J., 2012b. Assessment of bioburden on human and animal tissues: part 1–results of method development and validation studies. Cell Tissue Bank. 13, 129–138.
Kraft, L., Ribeiro, V.S.T., Wollmann, L.C., Suss, P.H., Tuon, F.F., 2018. Bioburden in transport solutions of human cardiovascular tissues: a comparative evaluation of direct inoculation and membrane filter technique. Cell Tissue Bank. 19, 447–454.
Lichtman, S.M., 2001. Bacterial [correction of baterial] translocation in humans. J. Pediatr. Gastroenterol. Nutr. 33, 1–10.
Loesche, W.J., 1969. Oxygen sensitivity of various anaerobic bacteria. Appl. Microbiol. 18, 723–727.
Malinin, T.I., Buck, B.E., Temple, H.T., Martinez, O.V., Fox, W.P., 2003. Incidence of clostridial contamination in donors' musculoskeletal tissue. J. Bone. Joint0. Surg. Br. 85, 1051–1054.
Martin, W.J., 1971. Practical method for isolation of anerobic bacteria in the clinical laboratory. Appl. Microbiol. 22, 1168–1171.
McCord, J.M., Keele Jr., B.B., Fridovich, I., 1971. An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. Proc. Natl. Acad. Sci. U. S. A. 68, 1024–1027.
Paolin, A., Trojan, D., Petit, P., Coato, P., Rigoli, R., 2017. Evaluation of allograft contamination and decontamination at the Treviso tissue Bank Foundation: a retrospective study of 11,129 tissues. PLoS One 12, e0173154.
Paolin, A., Montagner, G., Petit, P., Trojan, D., 2018. Contamination profile in allografts retrieved from multitissue donors: longitudinal analysis. Cell Tissue Bank. 19, 809–817.
Peterson, L.R., 1997. Effect of media on transport and recovery of anaerobic bacteria. Clin. Infect. Dis. 25 (Suppl. 2), S134–S136.
Sawa, B., Ribeiro, V.S.T., Kraft, L., Wollmann, L.C., Pegoraro, D., Suss, P.H., Tuon, F.F., 2019. Risk factors associated with contamination of allograft valves in a tissue bank. Cell Tissue Bank. 20, 87–94.
Schroeter, J., Wilkemeyer, I., Schiller, R.A., Pruss, A., 2012. Validation of the microbiological testing of tissue preparations using the BACTEC blood culture system. Transfus. Med. Hemother. 39, 387–390.
Spaulding, E.H., Vargo, V., Michaelson, T.C., Swenson, R.M., 1972. A comparison of two procedures for isolating anaerobic bacteria from clinical specimens. In: Balows, A., De Haan, R.M., Dowell jr.V.R., Guze, L.B. (Eds.), Anaerobic Bacteria: Role in Disease. Springfield.
Tally, F.P., Stewart, P.R., Sutter, V.L., Rosenblatt, J.E., 1975. Oxygen tolerance of fresh clinical anaerobic bacteria. J. Clin. Microbiol. 1, 161–164.
USP, 2017. Validation of alternative microbiological methods. In: United States Pharmacopeia, 40th ed. The United States Pharmacopeial Convention, Inc, Rockville.
van Belkum, A., Welker, M., Pincus, D., Charrier, J.P., Girard, V., 2017. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry in clinical microbiology: what are the current issues? Ann. Lab. Med. 37, 475–483.
van Kats, J.P., van Tricht, C., van Dijk, A., van der Schans, M., van den Bogaerdt, A., Petit, P.L., Bogers, A.J., 2010. Microbiological examination of donated human cardiac tissue in heart valve banking. Eur. J. Cardiothorac. Surg. 37, 163–169.