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Bacterial contamination rate and associated factors during bone and tendon allograft procurement from Spanish donors: exploring the contamination patterns

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SUMMARY

Background: Allograft contamination during extraction represents a major limiting factor for tissue bank availability. Contamination rates remain persistently high independent of the hospital, country or year considered.

Aim: To analyse the factors associated with contamination of bone and tendon samples extracted from Spanish donors.

Methods: Data for 1162 bone and tendon samples extracted from 102 donors between 2014 and 2017 were collected retrospectively from the hospital database. Descriptive statistics, potentially associated factors and correlation of contamination between samples extracted from different anatomical locations of the same donor were analysed.

Findings: In total, 227 (19.54%) of the extracted samples [131 (18.49%) bone samples and 96 (20.92%) tendon samples] rendered positive cultures and were discarded. Male sex [odds ratio (OR) 2.023; P=0.019], extraction of >10 samples per donor (OR 1.997; P<0.001) and extraction time >240 min (OR 1.755; P=0.001) were factors independently associated with a higher contamination rate. Meanwhile, the tissue sample type 'bone-patellar tendon-bone' was associated with a significantly lower contamination rate (OR 0.446; P=0.001). Significant correlation between certain localization of contaminated samples and the concordance of bacterial species was also observed.

Conclusion: Factors related to the extraction procedure, such as total extraction time, extraction sequence, number of samples extracted and anatomical location of extracted samples, play a major role in allograft contamination. Further optimization of procedures, guided by the contamination patterns analysed in this study, should help to increase tissue bank availability.

Introduction

Bone and tendon tissue allografts represent a fundamental part of the therapeutic arsenal of many surgical specialties, such as trauma, orthopaedic, spinal, oncologic and dental surgery [1–7].

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The increasing demand for these tissues poses a logistical and organizational challenge for transplant coordination departments and tissue banks, which must maintain increasingly demanding biosafety and quality standards. At present, a mounting interest in the optimization of organ and tissue procurement has led to the development of new strategies, such as living donor transplants and split liver transplantation [8].

In this context, contamination of samples during extraction represents an important obstacle to the overall efficiency of the donation process, as grafts with positive cultures are often discarded. Moreover, the added risk of transmission of potentially pathogenic agents has led the European Union [9,10] and countries such as the USA [11] to strictly reinforce their procurement, handling and storage protocols.

At present, there is no unanimity in the scientific literature about the predisposing factors for such contamination. The number of personnel in the operating theatre at the time of extraction [12–15], overall experience of the surgical staff regarding this type of procurement [13], time elapsed between death and extraction procedure [15,16], overall duration of extraction procedure [13,16], anatomical origin of tissue samples [12–14], total number of grafts extracted from each donor [13], lack of prophylactic antibiotic therapy in donors, and prolonged stay in the intensive care unit [16] have all been proposed as factors associated with a higher risk of graft contamination.

The variability of contamination rates between different centres, as well as their plateau over the last decade, suggests that there must be certain contamination factors that have not yet been adequately characterized, or that each hospital represents a complex environment in which the same factors interact in different ways. Moreover, information regarding the effectiveness of barrier and hygienic precautions taken during contemporary allograft procurement procedures to reduce contamination is currently lacking [17].

This study retrospectively analysed the factors potentially associated with contamination of bone and tendon samples extracted from donors at Hospital Clínico San Carlos, Madrid; an 800-bed hospital that has been performing organ and tissue extraction and implantation for more than 20 years. The results

Table I
Descriptive analysis and yearly distribution of the variables registered regarding donor and tissue sample characteristics

Variable			2014	2015	2016	2017	Total
Donor characteristics			*N*=27	*N*=24	*N*=25	*N*=26	*N*=102
	Sex	Male	25 (92.6)	19 (79.2)	23 (92)	22 (84.6)	89 (87.3)
		Female	2 (7.4)	5 (20.8)	2 (8)	4 (15.4)	13 (12.7)
	Age		47.74 (14–66)	51.04 (35–70)	45.40 (17–68)	51 (30–69)	48.77 (14–70)
	Type of donor	DCD	21 (77.8)	21 (87.5)	21 (84)	18 (69.2)	81 (79.4)
		DBD	6 (22.2)	3 (12.5)	4 (16)	8 (30.8)	21 (20.6)
Tissue samples			*N*=330	*N*=206	*N*=304	*N*=322	*N*=1162
Bone	Total number of bone samples		190 (57.6)	137 (66.5)	180 (59.2)	196 (60.9)	703 (60.5)
	Bone samples per donor		7.04 (4-13)	5.71 (3–10)	7.20 (1–18)	7.54 (1–14)	6.89 (1–18)
	Femoral condyle	Right	45 (13.6)	26 (12.6)	38 (12.5)	47 (14.6)	156 (13.4)
		Left	50 (15.2)	31 (15)	35 (11.5)	46 (14.3)	162 (13.9)
	Tibial plateau	Right	42 (12.7)	26 (12.6)	42 (13.8)	50 (15.5)	160 (13.8)
		Left	42 (12.7)	25 (12.1)	39 (12.8)	42 (13)	148 (12.7)
	Femoral diaphysis	Right	3 (0.9)	12 (5.8)	13 (4.3)	6 (1.9)	34 (2.9)
		Left	6 (1.8)	9 (4.4)	12 (3.9)	5 (1.6)	32 (2.8)
	Femoral head	Right	1 (0.3)	0 (0)	1 (0.3)	0 (0)	2 (0.2)
		Left	1 (0.3)	1 (0.5)	0 (0)	0 (0)	2 (0.2)
	Entire femur	Right	0 (0)	2 (1)	0 (0)	0 (0)	2 (0.2)
		Left	0 (0)	1 (0.5)	0 (0)	0 (0)	1 (0.1)
	Entire tibia	Right	0 (0)	2 (1)	0 (0)	0 (0)	2 (0.2)
		Left	0 (0)	2 (1)	0 (0)	0 (0)	2 (0.2)
Tendon	Total number of tendon samples		140 (42.4)	69 (33.5)	124 (40.8)	126 (39.1)	459 (39.5)
	Tendon samples per donor		5.19 (0-18)	2.88 (0–7)	4.96 (0–18)	4.85 (0–12)	4.50 (0–18)
	Achilles tendon	Right	29 (8.8)	16 (7.8)	24 (7.9)	31 (9.6)	100 (8.6)
		Left	32 (9.7)	17 (8.3)	24 (7.9)	26 (8.1)	99 (8.5)
	Bone-patellar tendon-bone	Right	31 (9.4)	15 (7.3)	26 (8.6)	28 (8.7)	100 (8.6)
		Left	21 (6.4)	15 (7.3)	24 (7.9)	26 (8.1)	86 (7.4)
	Hamstrings	Right	1 (0.3)	2 (1)	7 (2.3)	5 (1.6)	15 (1.3)
		Left	1 (0.3)	3 (1.5)	6 (2)	5 (1.6)	15 (1.3)
	Ankle tendons	Right	9 (2.7)	0 (0)	1 (0.3)	3 (0.9)	13 (1.1)
		Left	8 (2.4)	0 (0)	2 (0.7)	2 (0.6)	12 (1)
	Knee tendons	Right	3 (0.9)	1 (0.5)	6 (2)	0 (0)	10 (0.9)
		Left	5 (1.5)	0 (0)	4 (1.3)	0 (0)	9 (0.8)

DCD, donors after circulatory death; DBD, donors after brain death.
Results are shown as either *N* (%) or mean (range).

should help to define the margin of improvement that the process of bone and tendon donation offers in terms of minimizing contamination rates.

Methods

Data regarding all the bone and tendon grafts procured from donors after circulatory death (DCD) and brain death (DBD) registered at the study hospital between January 2014 and December 2017 were collected retrospectively from the hospital database. All bone and tendon grafts were extracted in operating theatres under sterile conditions by trauma and orthopaedic surgeons. The extraction procedure started 4–6 h after death and pre-operative skin preparation was performed separately for each extraction region using 10% povidone-iodine solution (Betadine; MEDA Pharma, Madrid, Spain). Skin preparation was performed following the same standards employed in orthopaedic surgery.

Contamination assessment was performed immediately after the tissue was procured by surface swabbing of the grafts. Grown bacteria were identified using matrix-assisted laser desorption/ionization mass spectrometry (Bruker Daltonik, Lepizig, Germany). The bacteria isolated in the tissue samples with positive cultures were categorized as low- or high-pathogenicity micro-organisms [12]. It was also specified when two or more species were isolated from one culture sample, as this information is relevant when antibiotic decontamination procedures are planned [18].

Descriptive statistics for all variables and bivariate analyses between tissue contamination and potentially associated factors were performed. Student's *t*-test and Mann-Whitney *U*-test were used to compare normally and non-normally distributed continuous variables, respectively. Comparison of proportions for categorical variables was performed by Fisher's exact test or Chi-squared test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for all valid associations. Multi-variate regression analyses were used to produce adjusted results for selected variables associated with contamination. A logistic regression model (backward stepwise) was performed using sample contamination as the dependent variable, and including those significantly ($P \leq 0.10$) associated on bivariate analysis as independent variables. The predictive power of the logistic regression model was expressed as the area under the receiver-operating characteristics curve.

Table III
Frequency of different bacterial species isolated in culture-positive bone and tendon grafts

Micro-organisms	Bone	Tendon	Total
Low pathogenicity	100 (76.3)	83 (86.5)	183 (80.6)
Bacillus spp.	1 (0.8)	4 (4.2)	5 (2.2)
Coagulase-negative staphylococci[a]	87 (66.4)	73 (76)	160 (70.5)
Corynebacterium spp.	4 (3)	0 (0)	4 (1.8)
Micrococcus spp.	7 (5.3)	4 (4.2)	11 (4.8)
Propionibacterium acnes	1 (0.8)	2 (2.1)	3 (1.3)
High pathogenicity	6 (4.6)	5 (5.2)	11 (4.9)
Bacteroides spp.	1 (0.8)	0 (0)	1 (0.4)
Escherichia coli	2 (1.5)	2 (2.1)	4 (1.8)
Staphylococcus aureus	2 (1.5)	1 (1)	3 (1.3)
Streptococcus spp.	1 (0.8)	2 (2.1)	3 (1.3)
Two or more species	25 (19.1)	8 (8.3)	33 (14.5)
All positive cultures	131 (100)	96 (100)	227 (100)

Results are shown as *N* (%).
[a] Includes *S. epidermidis, S. capitis, S. caprae, S. hominis, S. lugdunensis, S. oralis, S. pasteuri, S. pettenkofferi, S. simulans* and *S. warneri*.

In most cases, several bone and tendon grafts were recovered from each donor. Therefore, in order to explore the contamination patterns, correlations between a contaminated graft and other grafts extracted from the same donor that were also contaminated were analysed. Moreover, the same methodology was employed when grafts contaminated with the same micro-organism were considered. If the contamination source was common for all the grafts extracted from the same donor, it would be biologically plausible that the micro-organism isolated was the same in all samples. The quantitative value of the co-existence of contaminated samples from the same donor was expressed through the correspondent coefficient [Phi (Φ) and Cramer's V coefficients for binary and nominal variables, respectively]. Also, the probability of association happening by chance was calculated for each pair of grafts. In the analysis, only those tissue samples extracted from the majority of donors were considered (femoral condyle, tibial plateau, bone-patellar tendon-bone and Achilles tendon). The donor's body side was also considered, as a different surgeon performed the extraction of all samples from each body side.

Table II
Yearly distribution of bone and tendon graft contamination parameters

Variable		2014	2015	2016	2017	Total
Tissue samples		*N*=330	*N*=206	*N*=304	*N*=322	*N*=1162
Total number of contaminated samples (*N*)	Bone	37	23	33	38	131
	Tendon	36	8	24	28	96
	All samples	73	31	57	66	227
Contamination rate (%)	Bone	19.47	16.79	18.33	19.39	18.49
	Tendon	25.71	11.59	19.35	22.22	20.92
	All samples	22.12	15.05	18.75	20.5	19.54
Contaminated samples per donor [mean (range)]	Bone	1.37 (0–5)	0.96 (0–6)	1.32 (0–8)	1.46 (0–8)	1.28 (0–8)
	Tendon	1.33 (0–6)	0.33 (0–3)	0.96 (0–9)	1.08 (0–5)	0.94 (0–9)
	All samples	2.70 (0–6)	1.29 (0–6)	2.28 (0–17)	2.54 (0–8)	2.23 (0–17)

$P \leq 0.05$ was considered to indicate statistical significance. All statistical analyses were performed using SPSS Version 20 (IBM Corp., Armonk, NY, USA).

Results

Donor and tissue sample characteristics, along with yearly distribution, are summarized in Table I. All the results regarding sample contamination are summarized in Table II.

Table III summarizes the bacterial species isolated in bone and tendon samples. Factors potentially associated with sample contamination were explored through bivariate analysis, and the results are summarized in Table IV. Independent variables associated with sample contamination selected by the logistic regression model are summarized in Table V.

A graphical depiction of the association of contaminated samples between different anatomical locations is shown in Figure 1. Concordance of bacterial species between the contaminated samples extracted from each anatomical location is depicted in Figure 2.

Discussion

This study analysed the contamination rate of bone and tendon samples extracted from donors at a high-donor-volume Spanish hospital. In that sense, the contamination rates obtained are in concordance with the most recent contamination rates reported by other studies [14,16]. Due to the lack of sufficient tissues to meet demand, optimization of extraction-related procedures is of vital importance in order to reduce disposal of valid tissue samples due to bacterial contamination. Moreover, and due to the high costs associated with each step of the donation process, the development of contamination-reducing strategies is a priority in order to

Table IV
Bivariate analysis of predisposing factors associated with graft contamination

Variable		Culture-negative *N*=935	Contaminated *N*=227	OR (95% CI)	*P*-value
Year	2014	257 (27.5)	73 (32.2)	1.251 (0.914–1.711)	0.161
	2015	175 (18.7)	31 (13.7)	0.687 (0.455–1.038)	0.073
	2016	247 (26.4)	57 (25.1)	0.934 (0.669–1.303)	0.688
	2017	256 (27.4)	66 (29.1)	1.087 (0.789–1.498)	0.609
Donor characteristics					
Sex	Female	120 (12.8)	14 (6.2)	0.446 (0.252–0.792)	0.005
	Male	815 (87.2)	213 (93.8)	2.240 (1.262–3.976)	
Donor type	DCD	769 (82.2)	198 (87.2)	1.474 (0.964–2.253)	0.072
	DBD	166 (17.8)	29 (12.8)	0.679 (0.444–1.037)	
Age (years)	Mean (range)	46.59 (14–70)	46.13 (14–68)	–	0.548
	≤50	536 (57.3)	139 (61.2)	1.176 (0.874–1.582)	0.285
	>50	399 (42.7)	88 (38.8)	0.850 (0.632–1.144)	
Tissue sample					
Tissue type	Bone	572 (61.2)	131 (57.7)	0.866 (0.645–1.162)	0.338
	Tendon	363 (38.8)	96 (42.3)	1.155 (0.860–1.550)	
Anatomical piece	Femoral condyle	257 (27.5)	61 (26.9)	0.969 (0.699–1.344)	0.852
	Tibial plateau	249 (26.6)	59 (26)	0.968 (0.695–1.346)	0.845
	Femoral diaphysis	55 (5.9)	11 (4.8)	0.815 (0.419–1.583)	0.545
	Femoral head	4 (0.4)	0 (0)	–	0.419
	Complete femur	3 (0.3)	0 (0)	–	0.521
	Complete tibia	4 (0.4)	0 (0)	–	0.419
	Achilles tendon	146 (15.6)	53 (23.3)	1.646 (1.155–2.347)	0.006
	Bone-patellar tendon-bone	165 (17.6)	21 (9.3)	0.476 (0.295–0.768)	0.002
	Hamstrings	22 (2.4)	8 (3.5)	1.516 (0.666–3.451)	0.318
	Ankle tendons	17 (1.8)	8 (3.5)	1.973 (0.840–4.630)	0.112
	Knee tendons	13 (1.4)	6 (2.6)	1.926 (0.724–5.122)	0.182
Body side	Right	466 (49.8)	128 (56.4)	1.301 (0.972–1.742)	0.077
	Left	469 (50.2)	99 (43.6)	0.768 (0.574–1.029)	
Extraction process					
No. of samples extracted	≤10	335 (35.8)	51 (22.5)	0.519 (0.370–0.729)	<0.001
	>10	600 (64.2)	176 (77.5)	1.927 (1.373–2.705)	
Extraction time	≤240 min	429 (45.9)	82 (36.1)	0.667 (0.494–0.900)	0.008
	>240 min	506 (54.1)	145 (63.9)	1.499 (1.111–2.023)	
Time of day when extraction started	08:00–19:59	567 (60.6)	146 (64.3)	1.170 (0.865–1.582)	0.308
	20:00–07:59	368 (39.4)	81 (35.7)	0.855 (0.632–1.156)	
Time of day when extraction ended	08:00–19:59	252 (27)	74 (32.6)	1.311 (0.959–1.792)	0.089
	20:00–07:59	683 (73)	153 (67.4)	0.763 (0.558–1.043)	

DCD, donors after circulatory death; DBD, donors after brain death; OR, odds ratio; CI, confidence interval.
Results are shown as either *N* (%) or mean (range).

Table V
Adjusted variables associated with sample contamination rendered by the logistic regression model (Nagelkerke $R^2 = 0.071$: $P<0.001$; Hosmer & Lemeshow: $P=0.420$). The logistic regression model displayed an overall predictive accuracy of 65.4% (95% CI 61.7–69.1; $P<0.001$)

Variable	OR (95% CI)	*P*-value
Male sex	2.023 (1.124–3.641)	0.019
>10 samples extracted	1.997 (1.411–2.828)	<0.001
Extraction time >240 min	1.755 (1.273–2.420)	0.001
Bone-patellar tendon-bone	0.446 (0.274–0.726)	0.001
DBD	0.632 (0.398–1.002)	0.051
Right-body-side samples	1.335 (0.991–1.799)	0.058

DBD, donors after brain death; OR, odds ratio; CI, confidence interval.

guarantee the efficiency of the system. Therefore, any efforts directed towards contamination control should consider the associated factors. As a result, the present study analysed the factors potentially associated with bone and tendon sample contamination in the study hospital.

Although other authors have reported an association between donor's sex and sample contamination [15], this study found no biologically plausible explanation for this significant association, other than males were over-represented in the sample. No differences were observed between DCD and DBD. This supports the suitability of DCD for the extraction of bone and tendon tissues independent of the likely outdoor setting of these donors' deaths.

With respect to the tissue sample, no differences were observed in terms of tissue type or anatomical location, with the exception of the bone-patellar tendon-bone and the Achilles tendon. When the extraction process characteristics were studied, results showed a significant association between extraction time >240 min and higher probability of contamination. In that sense, other studies have also observed a higher risk of contamination with longer extraction times [13,16] and with the extraction of Achilles tendons [12,14].

A feature of the study hospital's extraction procedure is related to the body side that each surgeon occupies during procurement. Routinely, an associate surgeon occupies the right side of the operating table and a trainee surgeon occupies the left side of the operating table. Therefore, differences in surgical experience may have an impact on contamination rates, as other authors have observed [13]. Nevertheless, in the present study, no significant differences were observed with respect to the body side from which the sample was extracted and the contamination rate.

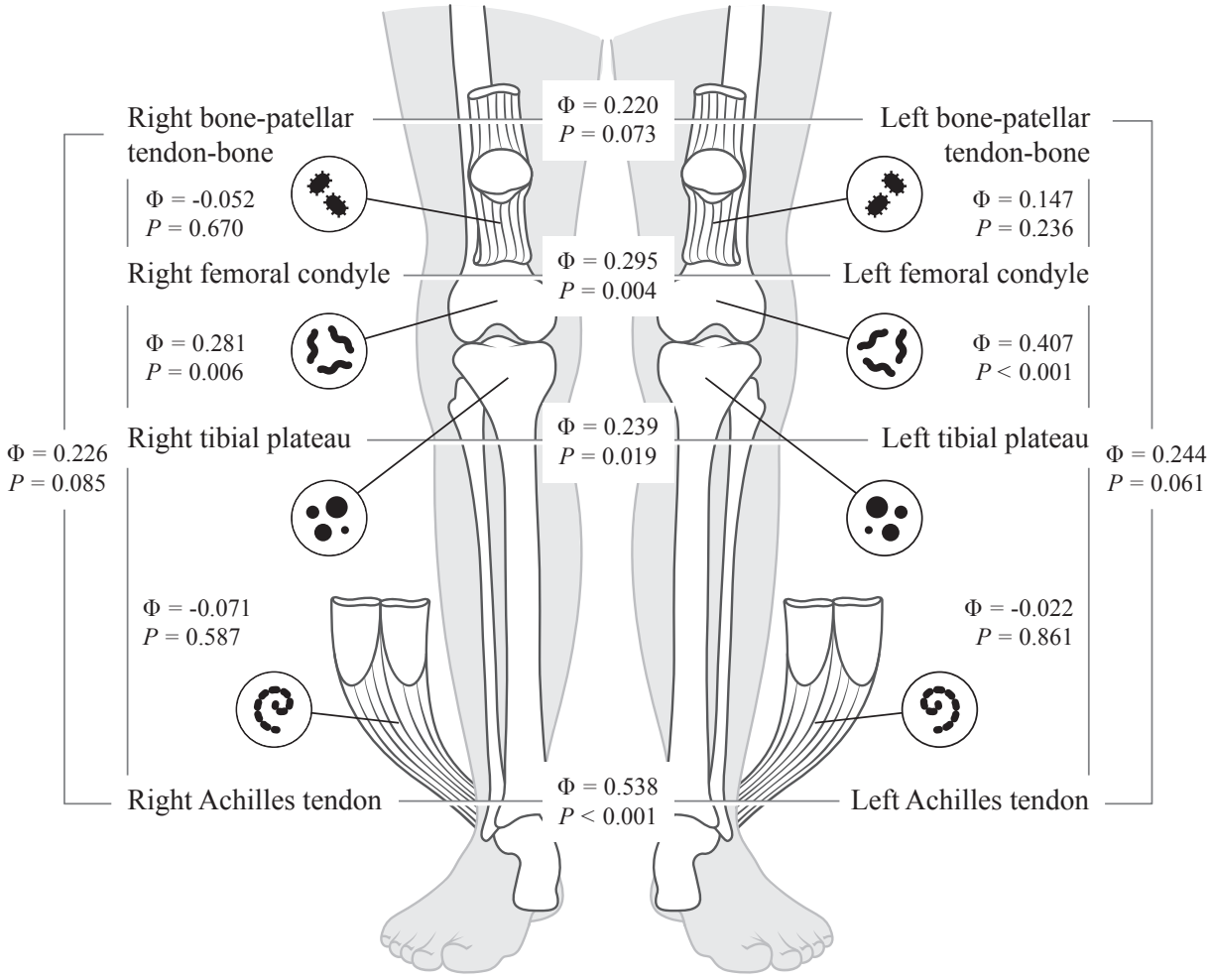

Figure 1. Analysis of the correlation of culture-positive grafts based on anatomical location. Φ, Phi coefficient.

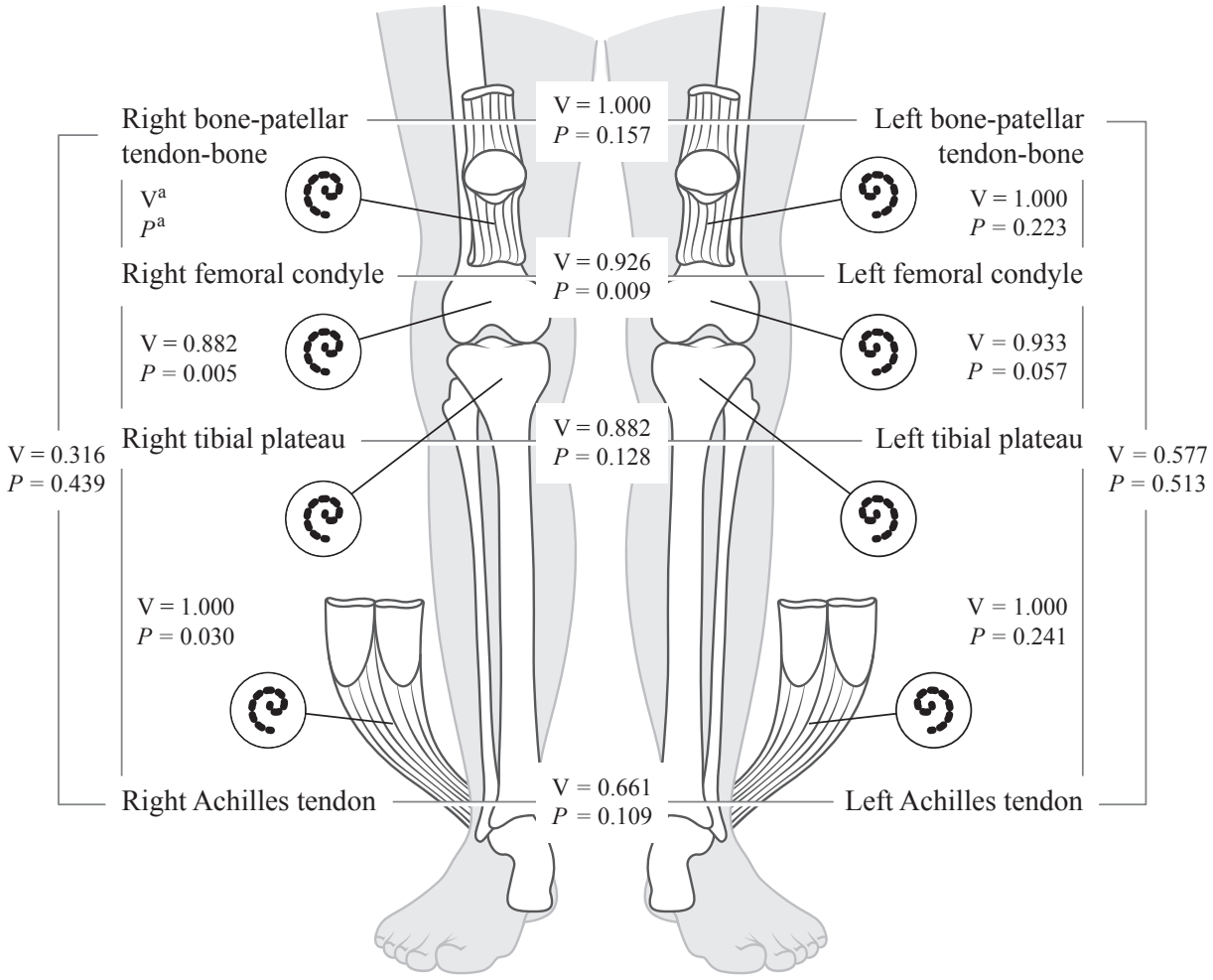

Figure 2. Analysis of the correlation of culture-positive grafts based on the bacterial species isolated. [a]Coefficient cannot be calculated as the variables are constant (only one species was isolated in all the samples from both locations). V, Cramer's V coefficient.

The present study also found an association between sample contamination and the total number of samples extracted from each donor. In that sense, establishing new contamination control checkpoints, such as changing the surgeon's gloves or employing a new set of sterilized instruments following the extraction of a certain number of samples, might contribute to more efficient procurement of samples.

This study also considered the time of day when extraction started and ended. It was hypothesized that the surgical team's fatigue or the number of hours they had already worked that day may have influenced meticulousness and attention to detail, leading to an increase in contamination rates. However, the results showed no significant differences.

To further explore the pattern in which sample contamination occurred, correlation between the anatomical locations that rendered culture-positive samples in each multitissue donor were analysed. The results showed that when one of the bone samples obtained from the femoral condyle was contaminated, there was a significantly higher probability of obtaining a contaminated sample from a nearby location (e.g. the respective tibial plateau or the contralateral femoral condyle). On the other hand, a contaminated sample extracted from a bone-patellar tendon-bone was not correlated with contamination of the bone samples located underneath, or contamination of the contralateral bone-patellar tendon-bone. This may be a consequence of the sequence order in which grafts are extracted. Also, it seems likely that prolonged tissue manipulation is an important factor contributing to sample contamination, as noted previously [14].

Regarding Achilles tendon extraction, contamination tended to occur in both sides, but was independent of contamination of more distant samples such as the tibial plateaus or the bone-patellar tendon-bone. The fact that it is difficult to clean feet thoroughly before starting the extraction procedure may be a contributing factor for the increased risk of contamination. Other authors have identified the Achilles tendon as a graft predisposed to contamination by skin commensals, attributing this to the detachment and subsequent contamination of surgical drapes [12,14].

In order to elucidate the existence of a common source of bacteria in the case of donors rendering multiple contaminated samples, correlation analysis of bacterial species was undertaken between the culture-positive samples extracted from different anatomical locations. The results showed that the species significantly coincided in the case of simultaneous contamination of both femoral condyles, and femoral condyles

and tibial plateaus on the right side. The reduced surgical field in which both extractions take place and the blood and bone splatter generated by osteotomy could explain this fact.

The finding that contamination correlation between anatomical locations was not significant between bone and tendon samples, despite their anatomical proximity in the case of the knee area, may reflect the impact of a contamination factor that has not been considered to date. As surgeons did not usually replace their surgical gloves during the extraction procedure, hand-borne contamination seems to be an unlikely bacterial source in the majority of cases. Meanwhile, different types of surgical instruments are used for bone and tendon extraction procedures, and both surgeons usually employ the same instrument across sides.

As the contamination pattern showed a likely influence of the sequence of extraction and tissue type on sample contamination, it is proposed that surgical instrument contamination by commensal skin flora may be a plausible explanation. Other authors have considered the surgical staff's skin flora as the main source of airborne bacteria that could deposit on to the graft surface [12]. The predominance of coagulase-negative staphylococci as contaminating agents of bone and tendon grafts among the literature [12,14,16] adds plausibility to this assertion. Nevertheless, no significant correlation was detected between contamination of the bone-patellar tendon-bone and the Achilles tendon, which would have been expected if airborne contamination was present. On the contrary, this could be explained by the fact that contamination of surgical instruments is time-dependent, as the bone-patellar tendon-bone is normally the first graft to be extracted and the Achilles tendon is usually the last. Other authors have found a higher risk of contamination for the last procured grafts, even if they were other than the Achilles tendon [13]. The authors believe that contamination of surgical instruments with the donor's commensal skin flora would better account for the results obtained for both anatomical location and bacterial species correspondence.

Cases of contamination by high-pathogenicity organisms, especially those normally found in respiratory and gastrointestinal tract flora (e.g. *Staphylococcus aureus*, *Streptococcus* spp., *Bacteroides* spp. and *Escherichia coli*), were scarce and tended to be limited to a group of grafts extracted from the same donor. From the authors' experience, respiratory droplets and defaecation due to sphincter relaxation prior to the bone and tendon graft extraction represent more frequent sources of this type of contamination than haematogenous dissemination, as proposed by other authors [12,14,19].

As a final consideration, it is important to note that careful interpretation of the direct cause—effect inferences obtained should be considered due to the retrospective observational nature of this study. The moderately good predictive power rendered by the regression model suggested that several other factors not considered in the analysis could have an impact on the sample contamination process. In order to explore the plausible nature of the hidden factors, an exploratory analysis based on correlation of the anatomical location of the contaminated tissues was performed. Future study designs should account for these limitations in order to further confirm the hypothesis presented in the present work.

In conclusion, the results suggest that factors related to the extraction procedure, such as total extraction time, extraction sequence, number of samples extracted and their anatomical location, play a major role in bone and tendon sample contamination. Further procedure optimization guided by the contamination patterns analysed in this study may help to increase tissue bank availability and the efficiency of the extraction process. All of these principles should be borne in mind before attempting ambitious tissue-banking projects in order to achieve good-quality standards.

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