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Dual-system microbiological screening to prevent fungal transmission in corneal grafts: a four-year eye bank retrospective study

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Abstract

Background

This study aimed to assess the effectiveness of a dual-system microbiological screening protocol implemented at the Emilia-Romagna Eye Bank in preventing fungal (specifically *Candida*) transmission in corneal grafts. Secondary objectives included identifying donor- and medical history-related risk factors for corneal contamination and strategies for managing "mate corneas" (paired corneas from a donor with one contaminated cornea).

Methods

A retrospective analysis was conducted of 2,273 corneal transplants performed and 3,138 donor corneas processed between January 2020 and December 2023. The dual-system screening used organ culture preservation, combined with turbidimetric and fluorimetric microbiological tests.

Results

Under the new protocol, no cases of postoperative Candida-related keratitis or endophthalmitis were observed among 2,255 transplanted corneas, representing a significant improvement over the previous 5 Candida-related complications in 5 transplants. Of the 3,138 donor donations analysed, 204 (6.5%) showed bacterial or fungal contamination, with 136 (4.3%) specifically positive for *Candida*. Significant risk factors for contamination included younger donor age, donation during the summer months, neoplastic disease, and longer death-to-explant intervals. The majority (62.3%) of mate corneas from donors with one contaminated cornea were discarded due to confirmed contamination or altered morphology.

Conclusions

The implemented dual-system microbiological screening protocol proved highly effective in preventing postoperative fungal infections among corneal transplant recipients. Donor age, seasonality, and post-mortem interval were identified as primary risk factors for corneal contamination. The findings support the wider adoption of comprehensive microbiological screening and organ culture techniques to improve transplant safety and optimise graft utilization.

1. Introduction

Corneal transplantation allows millions of individuals worldwide the possibility of recovering their eye sight (Salmon and Kanski 2020); however, infections in any phase of the procedure remain a major threat to graft survival (Eldestein et al. 2016). Microbial keratitis and endophthalmitis are the major

postoperative complications (Fontana et al. 2019; Chen et al. 2015), which can lead to graft failure and vision loss (Durand 2013).

While bacterial pathogens are the primary cause of infection, fungal infections account for approximately 20% of cases, with *Candida* albicans being the predominant species identified (Chen et al. 2015; Song et al. 2021).

Microbial infections related to contaminated donor tissues have decreased in recent years thanks to the development of increasingly efficient corneal preservation methods. The two most used techniques at present are hypothermic storage and organ culture. Hypothermic storage, widely practiced in the United States and in parts of Asia, involves preserving corneas at low temperatures (2–6° C) in solutions such as Optisol-GS. This approach reduces cellular metabolic activity, making storage feasible up to 14 days. Organ culture, more common in Europe, involves preserving tissues at temperatures close to physiological levels (31–37° C) for an extended period, typically 28 to 30 days (Eldestein et al. 2016; Fontana et al. 2007).

A study reported a significantly higher rate of positive postoperative corneoscleral rims with hypothermic storage (9.8%) compared to organ culture (1.3%). The main limitation of hypothermic storage is the shorter preservation time, which prevents multiple microbiological tests. Moreover, low temperatures suppress microbial growth, particularly fungal, allowing early detection of contamination. In contrast, organ culture maintains endothelial metabolism and enables comprehensive microbiological screening. Although more resource-intensive and technically demanding, organ culture better preserves endothelial viability and is associated with lower contamination rates (Fontana et al. 2007).

This study examined the first four years (2020–2023) of the implementation of a dual-system microbiological screening approach at the Emilia-Romagna Eye Bank, Italy. The primary outcome was to determine the risk of infectious keratitis, particularly *Candida* spp. infections, following corneal transplantation under a newly implemented microbiological testing protocol. Secondary outcomes were to identify potential donor-related or medical history-related risk factors associated with increased susceptibility to corneal contamination by any microorganisms and by *Candida* spp. specifically. Finally, the study aimed to explore strategies to preserve the “mate cornea” in cases where the paired cornea tested positive for contamination.

2. Methods and Materials

2.1 Population and setting

This retrospective study examined registry documentation for 2273 corneal transplants performed between January 2020 and December 2023, using corneas provided by the Emilia-Romagna Eye Bank (Bologna, Italy). The study was approved by the Institutional Review Board (Registration No. 512/2025/I.5/165) and adhered to the Declaration of Helsinki. Data from all donors recorded in the Eye Bank’s database during the same period were analysed retrospectively. The distribution of selected

variables within the sample was assessed, and their associations with bacterial and fungal contamination, specifically *Candida* spp., were evaluated.

The variables considered included sex, age, type of donation, history of alcoholism, anorexia, diabetes, septicaemia, bacteraemia, previous ocular surgery, hospital ward of death, place of tissue retrieval, cause of death, length of hospitalization, interval between death and explantation and season of the year (based on date of death). This analysis was subsequently repeated on the mate corneas, defined as the tissue retrieved from the contralateral eye of a donor whose paired cornea tested positive on microbiological analysis.

2.2 Protocols and microbiological tests in the Emilia-Romagna Eye Bank

As in the rest of Europe, the Emilia-Romagna Eye Bank stores the corneoscleral button in organ culture at 31° C for up to 30 days, in compliance with the guidelines approved by the National Transplant Centre on 14 September 2016. This protocol has already been described elsewhere (Fontana et al. 2007).

During organ culture preservation, all corneal storage media underwent microbiological testing to detect potential contamination: samples of cold storage medium were tested after seven days of corneal storage; corneal culture medium was tested after seven days of corneal storage and each time the cornea was handled; deswelling medium was tested 24h after transfer or after DSAEK or DMEK endothelial flap preparation (Fig. 1).

Corneas found to be contaminated during organ culture preservation, or those showing fungal contamination in the cold storage medium, were discarded, with only tissues free of contamination being used for transplantation (Wojcik et al. 2021).

Two microbiological screening methods are used: a turbidimetric system (HB&L Alifax System, Padua, Italy) and a fluorometric system (BD BACTEC FX System, New Cairo, Cairo, Egypt). The BACTEC FX System, validated for corneal preservation media, detects CO_2 via fluorescence every 10 minutes using sensors containing CO_2-sensitive compounds. Samples are inoculated into BACTEC Peds Plus/F (aerobic) and BACTEC Plus Anaerobic/F (anaerobic) bottles and incubated at 35 ± 1.5°C for seven days. The Alifax HB&L System uses multi-angle light scattering to assess turbidity and generate growth curves, enabling qualitative detection and quantitative estimation of viable bacteria (CFU/mL). Non-bacterial signals are eliminated through baseline correction. Samples are placed into aerobic, anaerobic, and fungal broths in sequence and incubated at 37°C for 48 hours in each.

In 2020, the Emilia-Romagna Eye Bank introduced an additional microbiological test on the corneal culture medium after 14 days of storage. This supplementary screening is reserved for specific cases: when the contralateral cornea microbiological tests are positive for fungi in the cold storage medium (to avoid an unnecessary discard of potentially healthy tissue), when the contralateral cornea microbiological tests are positive for microorganisms in the culture medium; when donor-related risk

factors are present (e.g., septic shock without blood culture results or clinical signs of localized fungal infection). This additional analysis is performed using the Alifax Uroquattro HB&L System for aerobic, anaerobic, and fungal detection. Corneal morphological evaluation is subsequently scheduled for day 16 or later, upon confirmation of a negative microbiological result.

2.3 Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD), while categorical variables are shown as absolute and relative frequencies. Differences between donations with and without at least one infected cornea (positive for bacteria, fungi, or *Candida*) were analysed using Pearson's χ^2 test and Student's t-test, as appropriate. Multivariable logistic regression analysis was performed to examine the association between infection and donor variables (sex, age, type of donation, cause of death, bacteraemia, previous eye surgery, days of hospitalization, interval between death and explant and season of death). The odds ratios (OR) and their 95% confidence intervals (CI) are reported. Mate corneas analysis was also conducted. Pearson's χ^2 test, Student's t-test, and ANOVA were used to compare differences in reasons for disposal.

All p-values are based on two-sided tests, with $P < 0.05$ considered statistically significant. The analyses were conducted with statistical software STATA SE, version 19.

3. Results

3.1 Populations

Of a total of 2,273 corneas retrieved in 2020–2023, 2,255 were used to perform transplants in Emilia-Romagna, 12 corneas were utilised in neighbouring regions and six corneas were sent to Rwanda. Lamellar keratoplasties accounted for most of the procedures, with 16 anterior lamellar keratoplasties (ALK), 270 Descemet membrane endothelial keratoplasties (DMEK) and 1066 Descemet stripping automated endothelial keratoplasties (DSAEK). Penetrating keratoplasty (PK) was performed in 894 cases. Stromal lenticules were used in 22 cases. Additionally, five corneas were employed for unspecified reasons such as urgent procedures, and cases in Rwanda and their surgical details were not formally recorded.

After transplant, thorough consecutive examinations focusing on the early detection and management of post-operative infectious complications were conducted. A minimum of one year of post-operative follow-up data are available for all corneal transplants carried out in Italy. No transplant performed with corneas from the Emilia-Romagna Eye Bank developed infections or showed evidence of microbial contamination during the observation period. The new protocol resulted in zero cases of *Candida*-related complications, whether keratitis or endophthalmitis, throughout the study period.

3.2 Analysis of corneal donations

The corneas were harvested from donors from the Emilia-Romagna region. A total of 6,503 harvested corneas from 3,262 donors were evaluated. 124 donors were excluded from the analysis due to the absence of an identification code or to incomplete medical history; therefore, only 3,138 corneas were analysed. Considering overall contamination rates, namely at least one of the two corneas is contaminated, with this additional test, only 204 out of 3,138 donations were positive (6.5%), of which 136 were positive for *Candida* spp (4.3%) (Table 1).

Table 1
Sociodemographic and clinical characteristics of donations with at least one cornea positive for bacteria or fungi

	Bacteria or fungi infection				Candida infection			
	OR adj*	**95% CI**		**p-value**	**OR adj***	**95% CI**		**p-value**
Sex								
Male	1.00				1.00			
Female	1.29	0.95	1.74	0.103	1.24	0.86	1.79	0.257
Age, years	0.98	0.96	0.99	0.000	0.98	0.96	0.99	0.014
Type of donation								
Corneas only	1.00				1.00			
Multi-organ	0.39	0.04	3.65	0.412	0.88	0.09	8.73	0.910
Multi-tissue	0.58	0.24	1.41	0.231	0.53	0.16	1.79	0.306
Neoplastic cause of death								
No	1.00				1.00			
Yes	1.20	0.88	1.65	0.245	1.46	0.99	2.14	0.054
Bacteraemia								
No	1.00				1.00			
Yes	0.89	0.54	1.45	0.636	1.09	0.62	1.91	0.769
Previous eye surgery								
No	1.00				1.00			
Yes	1.33	0.68	2.59	0.406	1.26	0.55	2.89	0.593
Days of hospitalization	1.00	1.00	1.01	0.466	1.00	0.99	1.01	0.821
Interval between death and explant								
0-6h	1.00				1.00			
6-12h	1.17	0.80	1.72	0.42	1.45	0.92	2.30	0.112
12-24h	1.53	1.07	2.19	0.021	1.53	0.97	2.40	0.067
> 24h	2.29	0.46	11.51	0.313	1.62	0.17	15.36	0.672
Season (date of death)								

	Bacteria or fungi infection				Candida infection			
	OR adj*	95% CI		p-value	OR adj*	95% CI		p-value
Sex								
Winter	1.00				1.00			
Spring	1.47	0.92	2.35	0.105	1.19	0.68	2.11	0.543
Summer	2.05	1.33	3.15	0.001	1.75	1.05	2.92	0.033
Autumn	1.31	0.81	2.10	0.269	1.22	0.70	2.13	0.493

*adjusted for sex, age, type of donation, neoplastic cause of death, bacteraemia, previous eye surgery, days of hospitalization, interval between death and explant, season of death

3.2.1 Microbiological positivity for bacteria or fungi

Females were more likely to test positive for microbial contamination (45.6% vs 39.1% among negatives), although this difference was not statistically significant (P = 0.068). The average age was lower in the positive group (65 ± 11.14 years) than in the negative group (68 ± 10.85 years) (P = 0.0002). A higher positivity percentage was observed in tissues from cornea-only donors (88.7% vs 81.7%), whereas multiorgan donors were less frequently positive (8.3% vs 14.6%). Patients who were hospitalized and died in Internal Medicine or Oncology wards showed the highest prevalence of positive corneal donations, 27.5% and 23%, respectively, compared to 22.8% and 17.1% among negatives. Particularly, patients who died from a neoplastic disease were found to be more likely to have microbial contamination (44.6% among positives and 34.8% among negatives). Donations made during the summer months were associated with a higher percentage of positive microbiological results compared to those made during the winter (36.3% vs 26.1%) (P = 0.005). Multivariable regression analysis confirmed this association, showing that the summer season was associated with a twofold higher probability of corneal contamination compared with winter (OR = 2.05, 95% CI 1.33–3.15, P = 0.001) (Fig. 2).

Overall, bacterial and fungal contaminations were also associated with age and the interval between death and explant. The regression analysis indicated that the probability decreased with increasing age (OR = 0.98, 95% CI 0.96–0.99, P < 0.001) and that explants performed 12–24 hours postmortem had a higher risk (OR = 1.53, 95% CI 1.07–2.19, P = 0.021) compared to those obtained within 0–6 hours (Fig. 3).

No statistically significant association between bacterial and fungal contamination and the other variables was observed (Table 2).

Table 2
Sociodemographic and clinical characteristics of donations with at least one cornea positive for bacteria or fungi (*missing data)

	Total (n = 3,138)		**Positive for bacteria or fungi**				
			No (n = 2,934)		**Yes (n = 204)**		
	N	**%**	**n**	**%**	**n**	**%**	**p-value**
Sex							
Male	1,897	60.45	1,786	60.87	111	54.41	0.068
Female	1,241	39.55	1,148	39.13	93	45.59	
Age, years **(mean and SD)**	68	10.89	68.14	10.85	65.23	11.14	0.000
Type of donation							
Corneas only	2,578	82.15	2,397	81.7	181	88.73	0.035
Multi-organ	445	14.18	428	14.59	17	8.33	
Multi-tissue	115	3.66	109	3.72	6	2.94	
Alcoholism*							
No	2,983	95.24	2,790	95.29	193	94.61	0.660
Yes	149	4.76	138	4.71	11	5.39	
Anorexia*							
No	3,117	99.46	2,914	99.45	203	99.51	0.916
Yes	17	0.54	16	0.55	1	0.49	
Diabetes*							
No	2,298	77.53	2,145	77.41	153	79.27	0.548
Yes	666	22.47	626	22.59	40	20.73	
Septicaemia*							
No	568	81.96	515	81.49	53	86.89	0.295
Yes	125	18.04	117	18.51	8	13.11	
Bacteraemia*							
No	2,799	89.37	2,615	89.31	184	90.20	0.691
Yes	333	10.63	313	10.69	20	9.80	

	Total (n = 3,138)		Positive for bacteria or fungi				
			No (n = 2,934)		Yes (n = 204)		
	N	%	n	%	n	%	p-value
Previous eye surgery*							
No	2,573	94.77	2,396	94.82	177	94.15	0.692
Yes	142	5.23	131	5.18	11	5.85	
Ward of death							
Intensive care	1,350	43.02	1,277	43.52	73	35.78	0.026
Internal medicine	726	23.14	670	22.84	56	27.45	
Oncology	548	17.46	501	17.08	47	23.04	
Other	514	16.38	486	1.56	28	13.73	
Place of collection*							
Mortuary	1,822	74.64	1,683	74.11	139	81.76	0.075
Hospital ward	602	24.66	573	25.23	29	17.06	
Operating room	14	0.57	12	0.53	2	1.18	
Home	3	0.12	3	0.13	0		
Cause of death							
Neoplastic	1,112	35.44	1,021	34.8	91	44.61	0.012
Cardiovascular	733	23.36	695	23.69	38	18.63	
Cerebrovascular	491	15.65	470	16.02	21	10.29	
Respiratory	347	11.06	317	10.80	30	14.71	
Infectious	105	3.35	102	3.48	3	1.47	
Trauma	85	2.71	80	2.73	5	2.45	
Other	265	8.44	249	8.49	16	7.84	
Days of hospitalization (mean and SD)	11	24.02	10.77	24.56	12.3	13.97	0.378
Interval between death and explant							
0–6h	1,063	33.88	1,001	34.12	62	30.39	0.624

	Total (n = 3,138)		Positive for bacteria or fungi				
			No (n = 2,934)		Yes (n = 204)		
	N	%	n	%	n	%	p-value
6–12h	840	26.77	786	26.79	54	26.47	
12–24h	1,063	33.88	986	33.61	77	37.75	
> 24h	172	5.48	161	5.49	11	5.39	
Season (date of death)							
Spring	744	23.71	695	23.69	49	24.02	0.005
Summer	840	26.77	766	26.11	74	36.27	
Autumn	765	24.38	720	24.54	45	22.06	
Winter	789	25.14	753	25.66	36	17.65	

SD: standard deviation

3.2.2 Microbiological positivity for *Candida* spp.

The average age was lower in the positive group (65 ± 11.3 years) compared to the negative group (68 ± 10.85 years). Microbial contamination was more frequent in patients who died from neoplastic diseases (47.1% among positives and 34.8% among negatives). In the multivariable regression analysis, significant associations between microbiological positivity for *Candida* spp. and both age and season were found, consistent with findings from corneas previously testing positive for bacteria or fungi. Death occurring during the summer months was linked to an increased probability of corneal contamination (OR = 1.75, 95% CI 1.05–2.92, P = 0.032), whereas the risk decreased with increasing age (OR = 0.98, 95% CI 0.96–0.99, P = 0.007). No statistically significant association between microbiological positivity for *Candida* spp. and any of the other variables was observed (Table 3).

Table 3
Sociodemographic and clinical characteristics of donations with at least one cornea positive for *Candida* (*missing data)

	Total (n = 3,070)		**Positive for *Candida***				
			No (n = 2,934)		**Yes (n = 136)**		
	n	**%**	**n**	**%**	**n**	**%**	**p-value**
Sex							
Male	1,861	60.62	1,786	60.87	75	55.15	0.182
Female	1,209	39.38	1,148	39.13	61	44.85	
Age, years (mean and SD)	68	10.89	68.14	10.85	65.03	11.30	0.001
Type of donation							
Corneas only	2,515	81.92	2,397	81.70	118	86.76	0.307
Multi-organ	443	14.43	428	14.59	15	11.03	
Multi-tissue	112	3.65	109	3.72	3	2.21	
Alcoholism*							
No	2,917	95.20	2,790	95.29	127	93.38	0.310
Yes	147	4.80	138	4.71	9	6.62	
Anorexia*							
No	3,050	99.48	2,914	99.45	136	100	0.388
Yes	16	0.52	16	0.55	0		
Diabetes*							
No	2,242	77.39	2,145	77.41	97	76.98	0.911
Yes	655	22.61	626	22.59	29	23.02	
Septicaemia*							
No	551	81.75	515	81.49	36	85.71	0.492
Yes	123	18.25	117	18.51	6	14.00	
Bacteraemia*							
No	2,736	89.30	2,615	89.31	121	88.97	0.900
Yes	328	10.70	313	10.69	15	11.03	

	Total (n = 3,070)		Positive for *Candida*				
			No (n = 2,934)		Yes (n = 136)		
	n	%	n	%	n	%	p-value
Previous eye surgery*							
No	2,511	94.79	2,396	94.82	115	94.26	0.788
Yes	138	5.21	131	5.18	7	5.74	
Ward of death							
Intensive care	1,324	43.13	1,277	43.52	47	34.56	0.075
Internal medicine	707	23.03	670	22.84	37	27.21	
Oncology	533	17.36	501	17.08	32	23.53	
Other	506	16.48	486	16.56	20	14.71	
Place of collection*							
Mortuary	1,774	74.48	1,683	74.11	91	81.98	0.060
Hospital ward	591	24.81	573	25.23	18	16	
Operating room	14	0.59	12	0.53	2	1.8	
Home	3	0.13	3	0.13	0		
Cause of death							
Neoplastic	1,085	35.34	1,021	34.80	64	47.06	0.020
Cardiovascular	716	23.32	695	23.69	21	15.44	
Cerebrovascular	486	15.83	470	16.02	16	11.76	
Respiratory	336	10.94	317	10.80	19	13.97	
Infectious	103	3.36	102	3.48	1	0.74	
Trauma	83	2.70	80	2.73	3	2.21	
Other	261	8.50	249	8.49	12	8.82	
Days of hospitalization (mean and SD)	11	24.18	10,77	24.56	11.65	13.65	0.675
Interval between death and explant							
0–6h	1,039	33.84	1,001	34	38	28.00	0.437

	Total (n = 3,070)		Positive for *Candida*				
			No (n = 2,934)		Yes (n = 136)		
	n	%	n	%	n	%	p-value
6–12h	826	26.91	786	26.79	40	29.41	
12–24h	1,034	33.68	986	33.61	48	35.29	
> 24h	171	5.57	161	5.49	10	7.35	
Season (date of death)							
Spring	726	23.65	695	23.69	31	22.79	0.122
Summer	813	26.48	766	26.11	47	34.56	
Autumn	752	24.50	720	24.54	32	23.53	
Winter	779	25.37	753	25.66	26	19.12	

SD: standard deviation

3.3 Mate cornea analysis

Of the 204 donations with at least one cornea positive for bacteria or fungi, 191 were included in the mate cornea analysis; similarly, 130 of the 136 *Candida*-positive donations were analysed (Table 4).

Table 4
Disposition of the mate corneas of donations with at least one cornea testing positive for bacteria or fungi and positive for *Candida*

	Positive for bacteria or fungi (n = 191)		Positive for *Candida* (n = 130)	
	n	%	N	%
Assigned	72	37.7	48	36.92
Disposed	119	62.3	82	63.08
Donor deemed unsuitable	*7*	*5.88*	*4*	*4.88*
Proven contamination	*64*	*53.78*	*49*	*59.76*
Altered morphology	*48*	*40.34*	*29*	*35.37*

3.3.1 Corneal donations with positive samples (all aetiologies)

Most of the mate corneas (62.3%) were discarded, with the main reasons being confirmed contamination (53.8%), altered morphology (40.3%), or donor unsuitability (5.9%). Moreover, none of the variables showed a statistically significant association with mate cornea disposition. However, there was a borderline significant trend between cornea disposal and the interval from death to retrieval: the disposal rate increased with time, rising from 24.4% within six hours postmortem to 41.2% in the 12–24 hours postmortem (P = 0.054) (Table 5).

Table 5
Mate cornea analysis: sociodemographic and clinical characteristics of corneal donations with bacteria or fungi positive samples. (*missing data)

	Total (n = 191)		Assigned (n = 72)		Discarded (n = 119)		p-value
	n	%	n	%	n	%	
Sex							
Male	102	53.40	42	58.33	60	50.42	0.288
Female	89	46.60	30	41.67	59	49.58	
Age, years (mean and SD)	64.73	11.24	63.00	11.49	65.78	10.99	0.0974
Type of donation							
Corneas only	170	89.01	62	86.11	108	90.76	0.319
Multi-organ	15	7.85	6	8.33	9	7.56	
Multi-tissue	6	3.14	4	5.56	2	1.68	
Alcoholism*							
No	180	94.24	68	94.44	112	94.12	0.925
Yes	11	5.76	4	5.56	7	5.88	
Anorexia							
No	190	99.48	71	98.61	119	100.00	0.197
Yes	1	0.52	1	1.39	0		
Diabetes*							
No	142	78.89	52	77.61	90	79.65	0.747
Yes	38	21.11	15	22.39	23	20.35	
Septicaemia*							
No	48	85.71	17	89.47	31	83.78	0.565
Yes	8	14.29	2	10.53	6	16.22	
Bacteraemia							
No	172	90.05	66	91.67	106	89.08	0.562
Yes	19	9.95	6	8.33	13	10.92	
Previous eye surgery*							

	Total (n = 191)		Assigned (n = 72)		Discarded (n = 119)		p-value
	n	%	n	%	n	%	
Sex							
No	168	94.92	64	95.52	104	94.55	0.774
Yes	9	5.08	3	4.48	6	5.45	
Ward of death							
Intensive care	66	34.55	23	31.94	43	36.13	0.143
Internal medicine	51	26.70	14	19.44	37	31.09	
Oncology	47	24.61	22	30.56	25	21.01	
Other	27	14.14	13	18.06	14	11.76	
Place of collection*							
Mortuary	129	80.63	46	80.70	83	80.58	0.556
Hospital ward	29	18.13	11	19.30	18	17.48	
Operating room	2	1.25	0		2	1.94	
Home	0	0.00	0		0		
Cause of death							
Neoplastic	91	47.64	38	52.78	53	44.54	0.540
Cardiovascular	34	17.80	12	16.67	22	18.49	
Cerebrovascular	19	9.95	7	9.72	12	10.08	
Respiratory	25	13.09	9	12.50	16	13.45	
Infectious	2	1.05	0	0.00	2	1.68	
Trauma	5	2.62	3	4.17	2	1.68	
Other	15	7.85	3	4.17	12	10.08	
Days of hospitalization (mean and SD)	12.44	14.22	13.76	15.63	11.64	13.30	0.3181
Interval between death and explant							
0–6h	60	31.41	31	43.06	29	24.37	0.054
6–12h	51	26.70	17	23.61	34	28.57	

	Total (n = 191)		Assigned (n = 72)		Discarded (n = 119)		p-value
	n	%	n	%	n	%	
Sex							
12–24h	69	36.13	20	27.78	49	41.18	
> 24h	11	5.76	4	5.56	7	5.88	
Season (date of death)							
Spring	46	24.08	19	26.39	27	22.69	0.091
Summer	69	36.13	18	25.00	51	42.86	
Autumn	41	21.47	19	26.39	22	18.49	
Winter	35	18.32	16	22.22	19	15.97	

SD: standard deviation

3.3.2 Corneal donations with positive samples (*Candida* spp.)

Most of the mate corneas (63.1%) were discarded for proven contamination (59.8%), altered morphology (35.4%), or donor unsuitability (4.9%). Patients who had been hospitalized in Internal Medicine wards showed a higher percentage of disposed corneas (35.4% disposed and 14.6% assigned) compared to other wards.

The disposal rate increased with longer intervals between death and explantation, rising from 20.7% within six hours postmortem to 40.2% at 12–24 hours postmortem. Among the discarded mate corneas, whether due to *Candida* contamination or to morphological alterations, most came from donors who had died of neoplastic diseases, with 51% of these corneas discarded because of *Candida spp* infection and 34.4% because of morphological changes (Table 6).

Table 6
Mate cornea analysis: sociodemographic and clinical characteristics of corneal donations with candida positive samples (*missing data)

	Total (n = 130)		Assigned (n = 48)		Discarded (n = 82)		p-value
	n	%	n	%	n	%	
Sex							
Male	71	54.62	27	56.25	44	53.66	0.775
Female	59	45.38	21	43.75	38	46.34	
Age, years (mean and SD)	64.78	11.39	63.81	10.90	65.34	11.69	0.4622
Type of donation							
Corneas only	113	86.92	42	87.50	71	86.59	0.986
Multi-organ	14	10.77	5	10.42	9	10.98	
Multi-tissue	3	2.31	1	2.08	2	2.44	
Alcoholism							
No	121	93.08	46	95.83	75	91.46	0.344
Yes	9	6.92	2	4.17	7	8.54	
Anorexia							
No	130	100.00	48	100.00	82	100.00	
Yes	0		0		0		
Diabetes*							
No	92	76.67	33	75.00	59	77.63	0.743
Yes	28	23.33	11	25.00	17	22.37	
Septicaemia*							
No	33	84.62	10	83.33	23	85.19	0.882
Yes	6	15.38	2	16.67	4	14.81	
Bacteraemia							
No	116	89.23	44	91.67	72	87.80	0.493
Yes	14	10.77	4	8.33	10	12.20	
Previous eye surgery*							

	Total (n = 130)		Assigned (n = 48)		Discarded (n = 82)		p-value
	n	%	n	%	n	%	
Sex							
No	111	94.87	41	93.18	70	95.89	0.520
Yes	6	5.13	3	6.82	3	4.11	
Ward of death							
Intensive care	42	32.31	15	31.25	27	32.93	0.036
Internal medicine	36	27.69	7	14.58	29	35.37	
Oncology	32	24.62	16	33.33	16	19.51	
Other	20	15.38	10	20.83	10	12.20	
Place of collection*							
Mortuary	86	91.13	31	79.49	55	82.09	0.441
Hospital ward	18	16.98	8	20.51	10	14.93	
Operating room	2	1.89	0		2	2.99	
Home	0		0		0		
Cause of death							
Neoplastic	64	49.23	27	56.26	37	45.12	0.678
Cardiovascular	18	13.85	4	8.33	14	17.07	
Cerebrovascular	15	11.54	5	10.42	10	12.20	
Respiratory	18	13.85	8	16.67	10	12.20	
Infectious	1	0.77	0		1	1.22	
Trauma	3	2.31	1	2.08	2	2.44	
Other	11	8.46	3	6.25	8	9.76	
Days of hospitalization (mean and SD)	11.99	13.84	14.06	14.84	10.78	13.16	0.193
Interval between death and explant							
0–6h	38	29.23	21	43.75	17	20.73	0.034
6–12h	38	29.23	12	25.00	26	31.71	

	Total (n = 130)		Assigned (n = 48)		Discarded (n = 82)		p-value
	n	%	n	%	n	%	
Sex							
12–24h	44	33.85	11	22.92	33	40.24	
> 24h	10	7.69	4	8.33	6	7.32	
Season (date of death)							
Spring	30	23.08	11	22.92	19	23.17	0.109
Summer	45	34.62	11	22.92	34	41.46	
Autumn	29	22.31	15	31.25	14	17.07	
Winter	26	20.00	11	22.92	15	18.29	

SD: standard deviation

4. Discussion

This study examined the implementation and monitoring in the Emilia-Romagna region of a protocol designed to reduce the risk of postoperative fungal infections, especially those caused by *Candida* spp. Among the 2273 corneal transplants included in the study, no case of microbial keratitis, *Candida* interface keratitis, or endophthalmitis was observed. These preliminary results confirm our expectations when compared with data from 2016–2019, the period before the protocol with the added safety steps was introduced in Emilia-Romagna. While the rate of infectious intra- or postoperative adverse events in the 2016–2019 period was indeed minimal (nine cases out of 1988 donations), it nevertheless decreased to zero, highlighting the importance of thorough microbiological testing of stored corneal materials.

A 2016 report by the Eye Bank Association of America (EBAA) documented 165 infectious complications among 354,930 corneal grafts between 2007 and 2014. Of these, fungal pathogens, mainly *Candida* spp., made up most of the culture-positive cases in both keratitis and endophthalmitis. The New York Eye and Ear Infirmary reported a 0.16% rate of fungal infection following PK out of 2466 grafts (de Oliveira Santos et al. 2018). The incidence of post-keratoplasty endophthalmitis ranges from 0.14% to 0.67%, occurring most commonly after penetrating keratoplasty. Culture concordance for *Candida* spp. was observed in 75% of mate corneas in endophthalmitis cases and 100% of those in keratitis cases when both donor and recipient cultures were available (Eldestein et al. 2016).

According to a study conducted at a tertiary eye care facility in South India between 2013 and 2022, the overall risk of infectious adverse events following keratoplasty was 0.11% (Chaurasia et al. 2024).

The United States and India predominantly use hypothermic storage (Wojcik et al 2021; Chaurasia et al. 2024). In the US, Optisol-GS is commonly used during storage; it contains antibacterial agents (gentamicin and streptomycin) but lacks antifungal supplementation. Ritterband et al. (2007) demonstrated that supplementation of Optisol-GS with 100 µg/mL voriconazole reduced fungal growth in donor tissues. Layer et al. (2014) corroborated this finding, showing that amphotericin B was more consistently effective in suppressing *Candida* spp. across a range of concentrations. The European standard recommends preserving organ cultures at 31–37° C for up to four weeks. During this time, microbiology tests are performed to identify microorganisms that have not been removed or suppressed by decontamination or antibiotics (Fontana et al. 2007; Wojcik et al. 2021).

Our study, therefore, contributes to the growing evidence highlighting the importance of preventive measures such as thorough donor screening, antifungal supplementation during hypothermic storage and strict microbiological screening to minimise infection risks. Furthermore, using organ culture has been shown to reduce the rates of positive fungal corneoscleral rim cultures and subsequent post-keratoplasty fungal infections (Fontana et al. 2007; Nelson et al. 1983). The absence of any infectious complication in our cohort underscores the clinical effectiveness of our current practices and suggests a potential model for others to follow. Specifically, our Eye Bank designed and implemented a longer, more rigorous security protocol for the mate cornea. These additional procedures ensured that only mate corneas deemed safe and suitable for transplantation were utilised, increasing the safety margin.

Regarding donor cornea contamination by bacteria or fungi and the donor variables we considered in this study, age, season and the interval between death and explant emerged as risk factors. Donors who were positive for bacteria or fungi were generally younger than those who were negative. A similar pattern has been observed in some studies, although several others found no meaningful correlation (Röck et al. 2017; Armitage et al. 2014; Ling et al. 2019; Khouani et al. 2014; Gruenert et al. 2017). Corneal retrieval during the summer months or within 12–24 hours postmortem was associated with an increased risk of contamination. Previous studies have observed this seasonal association, which can be explained by climatic seasonality. Younger age and the summer season were also linked to a higher risk of contamination by *Candida* spp.

Cornea donations from patients admitted to the Internal Medicine or Oncology wards showed a higher rate of microbial contamination than those from Intensive Care units or other hospital departments. As previously noted by Khouani *et al.* and Armitage *et al.*, microbial positivity was particularly common among donors who had died of neoplastic diseases (Armitage et al. 2014; Khouani et al. 2014). Moreover, when donations consisted solely of corneas, they were more likely to be contaminated, indicating a greater risk in this subgroup.

Sex, alcoholism, anorexia, diabetes, septicaemia, bacteraemia, previous eye surgery and the location where the cornea was collected were not associated with an increased risk of microbiological positivity.

Younger donors appear to be at higher risk of microbial contamination, possibly due to increased tissue metabolic activity, which may allow microorganisms to persist longer in the early post- mortem phase.

Moreover, sudden or non- hospital deaths, more common among younger individuals, often occur in less controlled environments, increasing the risk of surface contamination by environmental microbes. In contrast, donors who die from cancer or have prolonged hospital stays are typically exposed for extended periods to hospital settings, invasive procedures and repeated courses of antibiotics or immunosuppressive agents. These conditions can disrupt the body's normal microbial balance, encouraging colonisation by opportunistic or antifungal- resistant species, particularly *Candida* spp. Previous studies have documented similar associations, with higher contamination rates among donors with malignancies or extended hospital stays (Armitage et al. 2014; Khouani et al. 2014; Gruenert et al. 2017). The time elapsed between death and corneal retrieval has also been shown to be an important factor. When this interval exceeds 12 hours, the decline in tissue integrity and immune defences creates an ideal environment for microbial proliferation and migration within ocular structures (Röck et al. 2017; Ling et al. 2019). Seasonal differences have also been noted, with higher contamination rates during the summer months. Elevated temperatures and humidity during this period likely promote microbial activity and reduce the effectiveness of conventional cooling and disinfection methods. Comparable seasonal trends have been observed in other eye bank studies, emphasising the importance of considering seasonal climate when refining tissue recovery and preservation protocols (Röck et al. 2017; Ling et al. 2019). Collectively, these observations suggest that corneal contamination results from the interplay of donor- related, environmental and procedural factors. Enhancing disinfection measures and adapting retrieval practices, particularly during warmer seasons or for donors with systemic illnesses, could further improve tissue sterility and optimise outcomes in corneal transplantation.

Our analysis of mate corneas also provides valuable insight into the management of donor tissues when one cornea tests positive for microbial contamination. In our cohort, most mate corneas were discarded (approximately 63%), mainly due to confirmed contamination or altered morphology. This finding aligns with previous reports from the Eye Bank Association of America, where the mate cornea was transplanted in 86 of 99 endophthalmitis cases and in 62 of 66 keratitis cases, with infection developing in 24% and 19% of recipients, respectively (Eldestein et al. 2016; Keyhani et al. 2005). The relatively high discard rate observed in our study underscores the need for cautious decision-making when evaluating mate corneas from positive donors. However, our enhanced dual-system microbiological screening protocol offers a potential strategy to safely recover some of these tissues. By performing additional microbiological testing, particularly on day 14 of storage, our protocol enabled re-evaluation of mate corneas that would otherwise have been discarded, thereby minimising tissue waste while maintaining safety standards. Interestingly, most discarded mate corneas in *Candida*-positive cases originated from donors who had died from neoplastic diseases or who had had longer death-to-explant intervals, suggesting that systemic or procedural factors affecting one cornea likely influence the contralateral eye as well. This supports the concept that donor-related factors, rather than random contamination, are the predominant cause of bilateral positivity. Therefore, implementing extended testing protocols and individualized assessment criteria could provide a rational approach to safely increasing graft availability without compromising transplant safety.

The first limitation of this study is its retrospective design, which precludes establishing causal relationships between donor characteristics and microbial contamination. Although statistical associations were identified, unmeasured confounding factors such as variations in retrieval conditions, disinfection techniques, or staff experience may have influenced the results. Another limitation is the lack of an internal control group to demonstrate the effectiveness of the new protocol in reducing the risk of *Candida* infection. However, we did compare our results with those reported in the literature. It is important to note that factors beyond storage methods may also affect comparability. Third, the overall incidence of contamination and infection was low, reflecting the effectiveness of the current preservation and screening systems, but limited statistical power for subgroup analyses, particularly for *Candida*-specific outcomes. Multicentre studies incorporating molecular diagnostic tools and standardized data collection would help validate our findings and refine donor-screening protocols to further reduce the risk of contamination.

5. Conclusions

In conclusion, the zero incidence of *Candida* postoperative keratitis in this regional cohort demonstrates the effectiveness of the Emilia-Romagna Eye Bank's new protocol. Based on the historical incidence rate, we expected between 1 and 4 cases of infection, not zero. Donor age and seasonality were linked to the overall and *Candida*-specific rates of corneal contamination. Postmortem intervals were associated with a higher risk of general contamination. Our results support the wider adoption of organ culture techniques and comprehensive microbiological screening protocols to further improve transplant safety.

Declarations

Funding:

This research received no external funding.

Review Board Statement:

All procedures were conducted in accordance with the World Medical Association's Declaration of Helsinki.

The authors confirm that all specimens were procured in accordance with the WHO Guiding Principles on Human Cell, Tissue, and Organ Transplantation.

Ethical approval was granted by the Institutional Review Board (Registration No. 512/2025/I.5/165) of CET-CEROM (Ethical Committee of Romagna)

Informed Consent Statement:

The authors declare that informed, voluntary consent was obtained from the donors or their next of kin, and that no organs or tissues were sourced from executed prisoners or prisoners of conscience.

Conflicts of Interest:

The authors declare no conflicts of interest.

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Formal analysis: M.A.M

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Data curation: N.d'G, M.M

Writing - original draft preparation: A.L, F.C, E.N,

Writing - review and editing: A.M, L.F

Visualization: A.M,

Supervision: D.D, L.F

Project administration: R.C, P.B

All authors have read and agreed to the published version of the manuscript.

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Figures

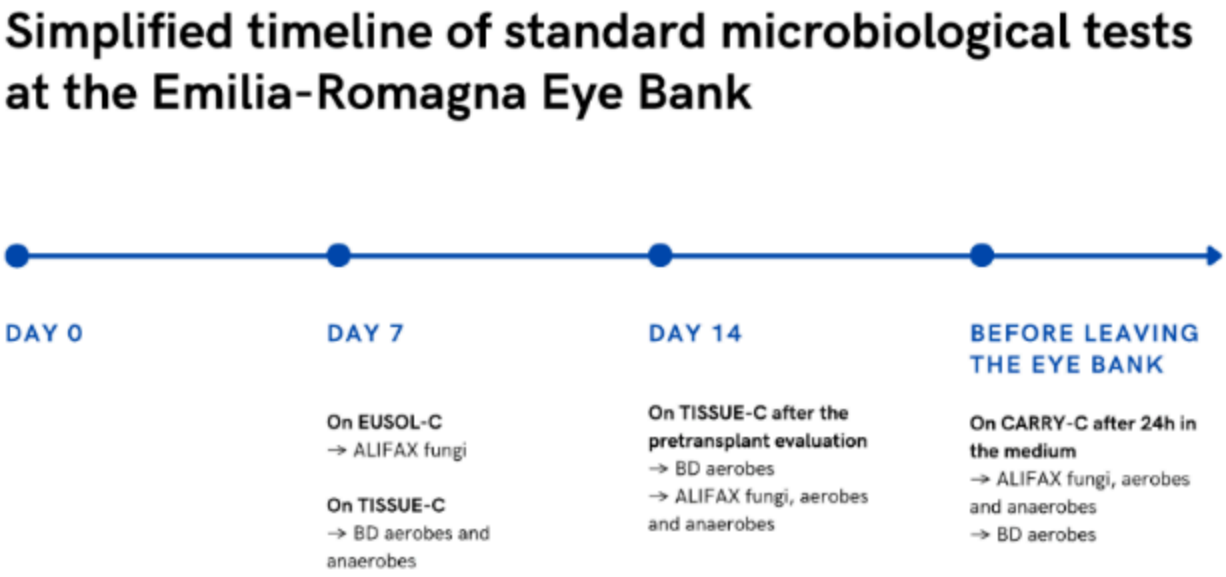


Figure 1

Simplified timeline of standard microbiological tests at the Emilia-Romagna Eye Bank (This figure should be printed in colour.)

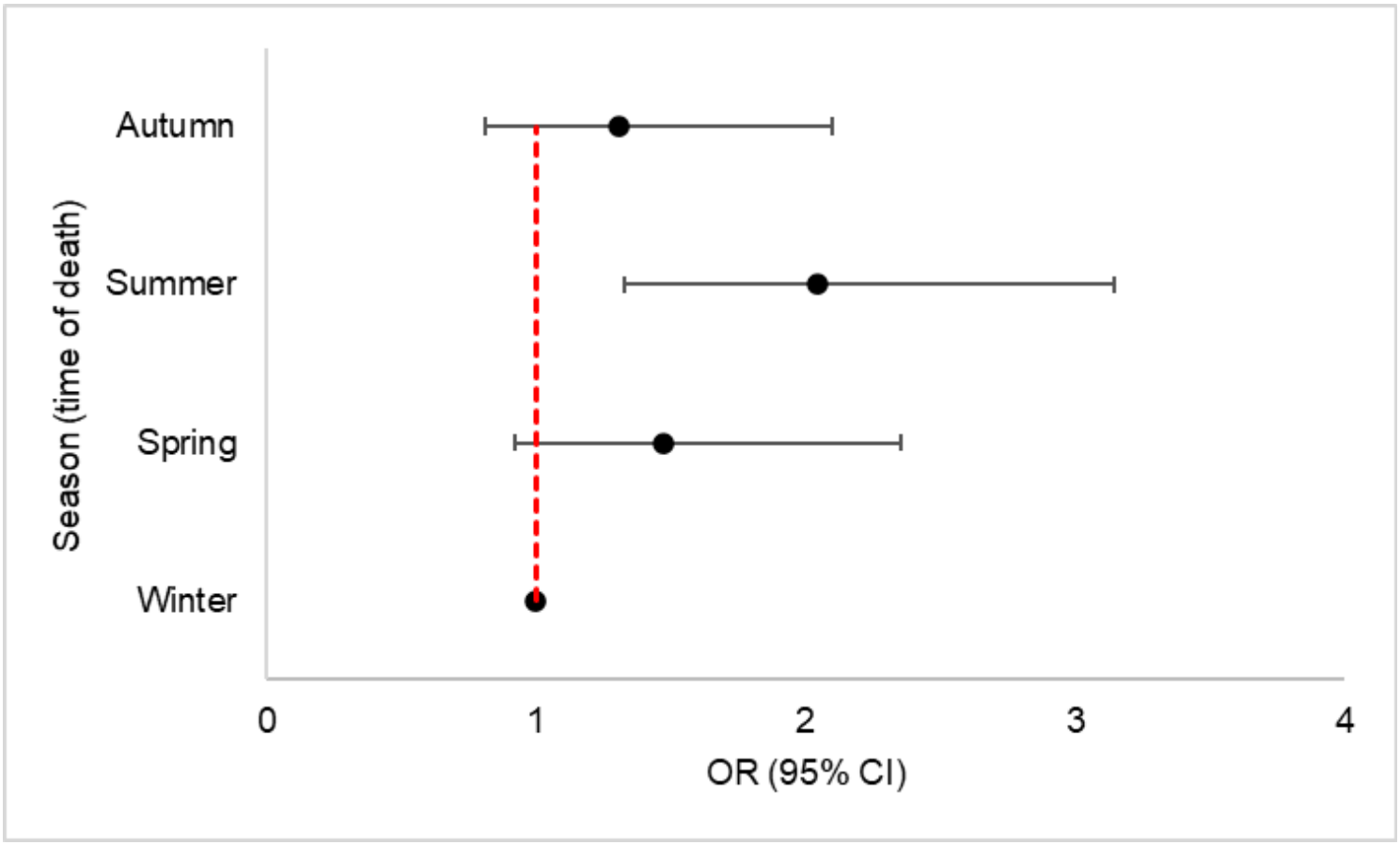


Figure 2

Bacteria or fungi infection adjusted* OR (95% CI) for season (time to death).

OR and 95% CI are represented by the dot and the horizontal interval respectively

**adjusted for sex, age, type of donation, cause of death, bacteraemia, previous eye surgery, days of hospitalization, interval between death and explant and season of death*

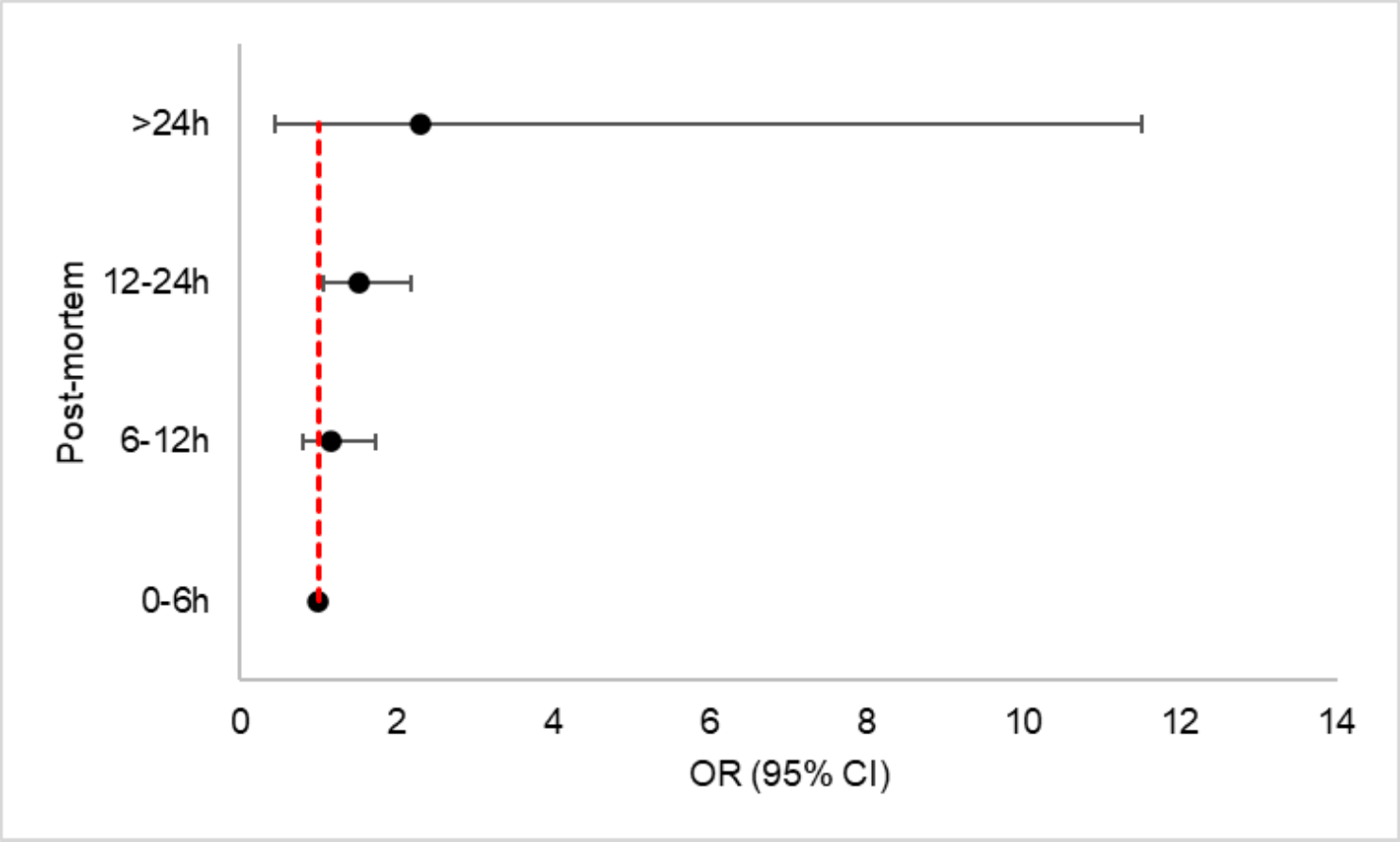


Figure 3

Bacteria or fungi infection adjusted* OR (95% CI) for post-mortem time intervals.

OR and 95% CI are represented by the dot and the horizontal interval respectively

**adjusted for sex, age, type of donation, cause of death, bacteraemia, previous eye surgery, days of hospitalization, interval between death and explant and season of death*