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# Maiden Experience in Processing, Preservation and Banking of Aortic Homograft from Human Cadaver in Bangladesh

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**Abstract**

**Background**: In Bangladesh, the prospect of using homograft is immense. However, homograft banking is not available in this country until now. The aim of this study was to evaluate the processing, preservation and banking of aortic homograft from human cadaver in Bangladesh. **Material & Methods:** This observational study was conducted in Department of Cardiac Surgery, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, Bangladesh from July 2016 to June 2018. The study included 30 procured hearts from adult unclaimed deceased individuals, who underwent autopsy within 24 hours of death at the Department of Forensic Medicine, Dhaka Medical College Hospital, Dhaka, Bangladesh, were categorized into two groups: group-A: 15 homograft valves prepared as Aortic Sleeve (AS), and group-B: 15 homograft valves prepared as Aortic Conduit (AC). **Results:** The Mean ± SD age in Group-A and Group-B was 34.27 ± 8.16 years and 34.20 ± 8.22 years, respectively. Gender distribution in Group-A included 11 (73.33%) males and 4 (26.67%) females, while Group-B had 12 (80%) males and 3 (20%) females. Morphological abnormalities were minimal, with 1 (6.67%) valve in Group-A and 2 (13.33%) in Group-B showing leaflet incompetency. Microbiological analysis revealed statistically significant differences in contamination rates before antibiotic treatment ($p<0.05$), while after antibiotic treatment, both groups demonstrated effective decontamination. Cryopreservation exhibited a 100% success rate in maintaining sterility, with no instances of contamination in both groups ($p>0.05$). **Conclusions:** The current study concludes that it is possible and effective to procure aortic homograft from human cadaver for aortic replacement surgery.

**Keywords**:- Processing, Preservation, Banking, Aortic Homograft, Human Cadaver, and Bangladesh.

## INTRODUCTION

The concept of aortic homograft has evolved significantly over the past few decades, offering a viable option for various cardiovascular surgical procedures. Aortic homograft, primarily used in aortic valve replacement and reconstruction of the aorta, have been favored due to their excellent biocompatibility, lower incidence of thromboembolic events, and resistance to infection compared to synthetic prostheses (Barratt-Boyes, 1964; O'Brien et al.,

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1987).[1,2] The use of homograft in cardiovascular surgery has been reported to have superior outcomes in specific clinical scenarios, such as infective endocarditis (Musci et al., 2010).[3] The process of harvesting, processing, and preserving these homografts is intricate and requires adherence to stringent protocols to ensure tissue viability and safety. The preservation of aortic homografts typically involves cryopreservation, a technique that has been refined over the years to optimize tissue integrity and function post-implantation (Brockbank et al., 1999).[4] Recent advancements in cryopreservation have focused on improving the longevity and durability of these grafts (The Future of Homografts, 1987).[5] In the context of Bangladesh, establishing a homograft bank poses unique challenges and opportunities. The country's tropical climate, resource constraints, and the need for specialized training in tissue processing and preservation are significant considerations. However, the establishment of such a facility could significantly enhance the quality of cardiovascular care in the region, providing access to high-quality graft materials for aortic reconstruction surgeries. The concept of tissue banking is not new, and various models exist globally that can be adapted to the Bangladeshi context. For instance, the Barcelona Tissue Bank has made significant strides in enhancing tissue quality and safety through the use of new antibiotic cocktails and changing microbiological control strategies (Last twenty-years activity of cardiovascular tissue banking in Barcelona, 2023).[6] These experiences can provide valuable insights into establishing a homograft bank in Bangladesh. Furthermore, the clinical application of aortic homografts has been well documented. Studies have shown the effectiveness of cryopreserved iliac artery and vein homografts in liver transplantation and carotid artery reconstruction (Banking of cryopreserved iliac artery and vein homografts, 2014).[7] Additionally, the management of graft infections, particularly in the ascending aortic position, has been revolutionized by the use of homografts, offering a solution to a previously challenging clinical scenario (Homografts for the management of graft infections, 2014).[8] The sterilization and storage of these grafts are also critical components of the banking process. Techniques such as electron beam energy and cold storage at -70°C have been explored for aortic valve homografts, showing promising results in reducing the incidence of arterial emboli (Results of Aortic Valve Replacement Utilizing Irradiated Valve Homografts, 1969).[9] Imaging techniques play a crucial role in both pre- and postoperative assessments of aortic root surgeries involving homografts. Advanced imaging modalities provide critical insights into the anatomy and pathology, aiding in surgical planning and postoperative monitoring (Pre- and Postoperative Imaging of the Aortic Root, 2016).[10] The biocompatibility of homografts is another area of ongoing research. Studies have shown that decellularized xeno-/allogeneic matrices exhibit enhanced vascular biocompatibility in rodent models, suggesting potential avenues for improving graft acceptance and function (Enhanced vascular biocompatibility, 2017).[11] The establishment of an aortic homograft bank in Bangladesh represents a significant step forward in the field of cardiovascular surgery in the region. Drawing on global experiences and advancements in tissue processing, preservation, and clinical application, this study aims to lay the groundwork for a sustainable and effective homograft banking system in

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Bangladesh. The successful implementation of this project has the potential to significantly improve the outcomes of cardiovascular surgeries in the country, ultimately contributing to better patient care and health outcomes.

**Objectives**

To evaluate the processing, preservation and banking of aortic homograft from human cadaver in Bangladesh.

## MATERIAL AND METHODS

This observational study was conducted in Department of Cardiac Surgery, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, Bangladesh from July 2016 to June 2018 (2 years duration). Total 30 procured hearts from the adult unclaimed dead bodies those underwent autopsy within 24 hours of death at Department of Forensic Medicine, Dhaka Medical College Hospital, Dhaka, Bangladesh were included in this study. The study subjects were divided into two groups where group-A: 15 homograft valves that were prepared as Aortic Sleeve (AS) consisting the aortic valve with intra-pericardial part of ascending aorta and group-B: 15 homograft valves that were prepared as Aortic Conduit (AC) or valved conduit consisting the aortic valve, ascending aorta, branched aortic arch and proximal 2 cm of descending aorta. Collection of cadaveric heart was done aseptically in operating room. The heart enclosed in pericardium along with the great vessels (aorta up to the proximal descending aorta, pulmonary trunk with its bifurcation and the proximal portion of the venae cavae) was removed from the human cadaver by median sternotomy. In the tissue-processing laboratory under strict aseptic condition, the dissection of the valve was done with meticulous surgical techniques. Ethical clearances from both Institutional Review Board (IRB), BSMMU and Department of Forensic Medicine, Dhaka Medical College was taken prior to commencement of the study. After cleaning, the data were entered into computer and statistical analysis of the results being obtained by using windows-based computer software devised with Statistical Packages for Social Sciences version 21. The findings of the study were presented in different tables and figures. Categorical variables were analyzed by Chi-Square test and Fisher exact test and continuous variables were analyzed by 't' test. Tests with p-value ≤ 0.05 were considered as statistically significant.

**Inclusion criteria**

- Adult dead bodies (aged from 18-55 years) of both sexes.
- Underwent autopsy within 24 hours of death.

**Exclusion Criteria**

- Mutilated dead bodies or >24 hours of death.
- Known septic or communicable disease.
- History of known connective tissue diseases, e.g. Marfan's syndrome.
- History or evidence of other cardiac diseases.
- Any visible morphological or developmental anomaly.

## RESULTS

[Table 1] shows distribution of subjects by age, sex and Body surface area (BSA). The Mean ± SD age in Group-A and Group-B was 34.27 ± 8.16 years and 34.20 ± 8.22 years respectively. The mean difference of age (in years) was

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statistically non-significant (p>0.05). Mean ± SD BSA for Group-A was 1.71 ± 0.17 m2 for male and 1.55 ± 0.1 m2 for female. Mean ± SD BSA for Group-B was 1.69 ± 0.14 m2 and 1.68 ± 0.03 m2 for male and female respectively. The difference of mean value of BSA was statistically non-significant (p>0.05). In Group-A, 11 (73.33%) subjects were male and 4 (26.67%) were female. In Group-B, there were 12 (80%) male and 3 (20%) female. The mean difference of gender was statistically non-significant (p>0.05). [Table 2] shows comparison of morphological abnormalities between two groups before cryopreservation. In Group-A, only 1 (6.67%) valve showed leaflet incompetency. In Group-B, total 2 (13.33%) valves showed leaflet incompetency and 1 (6.67%) valve encountered traumatic damage during dissection. Other morphological abnormalities were absent in both groups. Difference in the means of morphological abnormalities were statistically non-significant (p>0.05). [Table 3] shows comparison of morphological abnormalities between two groups after cryopreservation. In both groups, all valves with apparently healthy morphology before cryopreservation showed no morphological abnormalities after cryopreservation. Difference in the means of morphological abnormalities were statistically non-significant (p>0.05). [Table 4] shows, in Group-A, 2 (13.33%) valves were found contaminated with presence of organisms whereas reminder 13 (86.67%) valves showed no contamination before antibiotic treatment. In Group-B, presence and absence of organisms before antibiotic treatment were 7 (46.67%) and 8 (53.33%) respectively. Difference in the means of presence of organisms before antibiotic treatment between two groups was statistically significant (p<0.05). In Group-A, after antibiotic treatment, only 1 (6.67%) valve was found contaminated whereas reminder 14 (93.33%) valves showed no contamination. In Group-B, presence and absence of organisms after antibiotic treatment were 1 (6.67%) and 14 (93.33%) respectively. Difference in the means of presence of organisms after antibiotic treatment between two groups was statistically non-significant (p>0.05). [Figure 1] demonstrates a Bar diagram showing effect of antibiotic treatment on contaminated valves. In Group-A, 2 contaminated valves underwent antibiotic treatment, resulting in the retention of the organism in 1 case and successful decontamination in another. On the other hand, Group-B comprised 7 contaminated valves subjected to antibiotic therapy. Among these, 1 valve retained the organism post-treatment, while 6 valves were successfully decontaminated. Overall, the study found that out of the total 9 valves treated with antibiotics, 2 valves retained the organism after treatment (1 in each group), while 7 valves were effectively decontaminated (1 in Group-A and 6 in Group-B). These results provide valuable insights into the efficacy of antibiotic interventions in the context of contaminated valves. [Figure 2] presents the frequency of organisms isolated by microbiological culture before and after antibiotic treatment. Before the initiation of antibiotic therapy, Pseudomonas aeruginosa was the most frequently isolated organism, identified in 5 cases. Staphylococcus aureus and Streptococcus epidermidis were also prevalent, with 2 cases each. Candida albicans was not detected in any of the valves prior to antibiotic treatment. Following antibiotic intervention, pseudomonas aeruginosa decreased from 5 to 2 cases, indicating a partial response to the antibiotic

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treatment. Importantly, Staphylococcus aureus and Streptococcus epidermidis were completely eradicated, with no instances of these organisms being isolated post-treatment. Additionally, Candida albicans remained absent in the valves after antibiotic therapy. Table-V shows, both groups retained sterility (absence of organism) after cryopreservation. Difference in the means of presence and absence of organisms after cryopreservation between two groups was statistically non-significant ($p>0.05$). [Figure 3] demonstrates the 3D bar diagram showing effectiveness of cryopreservation in relation to microbiology. In both groups, a total of 22 sterile valves were cryopreserved. In Group-A, none of the cryopreserved valves became contaminated, showcasing a 100% success rate in maintaining sterility. Correspondingly, all 12 valves in Group-A remained sterile after cryopreservation. In Group-B, a similar outcome was observed, with none of the cryopreserved valves becoming contaminated. Out of the 10 valves in Group-B, all maintained sterility through the cryopreservation process. Overall, when considering both groups collectively, there were no instances of cryopreserved valves becoming contaminated. The effectiveness of cryopreservation in preserving sterility is evident, as all 22 valves across the two groups remained free from microbial contamination.


**Image 1:** Picture showing preserved aortic homograft

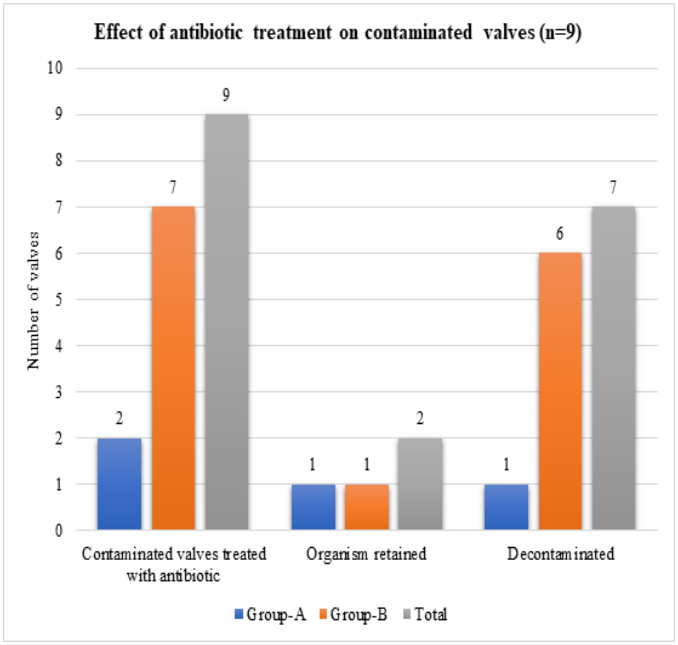


**Figure 1:** Bar diagram showing effect of antibiotic treatment on contaminated valves.

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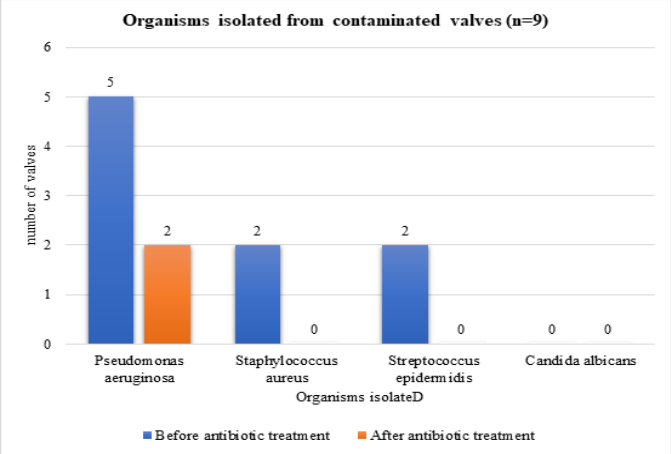


**Figure 2:** Bar diagram showing frequency of organisms isolated by microbiological culture before and after antibiotic treatment.

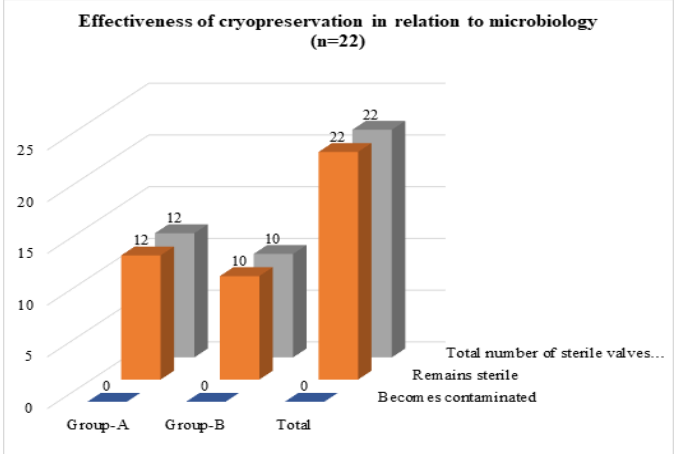


**Figure 3:** 3D bar diagram showing effectiveness of cryopreservation in relation to microbiology.

**Table 1:** Demographic characteristics of the study subjects (N=30)

| **Variables** | **Group-A (n=15)** | **Group-B (n=15)** | **p value** |
|---|---|---|---|
| *Age (in years) | | | |
| Mean± SD | 34.27 ± 8.16 | 34.20 ± 8.22 | $0.98^{ns}$ |
| *BSA (m2) | | | |
| Male (Mean± SD) | 1.71 ± 0.17 | 1.69 ± 0.14 | $0.83^{ns}$ |
| Female (Mean± SD) | 1.55 ± 0.1 | 1.68 ± 0.03 | $0.13^{ns}$ |
| **Sex | | | |
| Male (n, %) | 11 (73.33%) | 12 (80%) | $1^{ns}$ |
| Female (n, %) | 4 (26.67%) | 3 (20%) | |

*Data was analyzed using unpaired t-test and expressed as Mean ± SD
**Data was analyzed using Fisher exact test and expressed as frequency
**Figures in parentheses denote corresponding percentage
n = Total number of subjects
ns = non-significant

**Table 2:** Comparison of morphological abnormalities between two study groups before cryopreservation (N=30)

| **Variables** | **Group-A (n=15)** | **Group-B (n=15)** | **p-value** |
|---|---|---|---|
| Congenital anomaly | | | |
| Present | 0 | 0 | $1^{ns}$ |
| Absent | 15 (100%) | 15 (100%) | |
| Vegetation | | | |
| Present | 0 | 0 | $1^{ns}$ |

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| | | | |
|---|---|---|---|
| Absent | 15 (100%) | 15 (100%) | |
| Traumatic damage | | | |
| Present | 0 | 1 (6.67%) | $1^{ns}$ |
| Absent | 15 (100%) | 14 (93.33%) | |
| Visible color change | | | |
| Present | 0 | 0 | $1^{ns}$ |
| Absent | 15 (100%) | 15 (100%) | |
| Valve annular diameter shrinkage/dilatation | | | |
| Present | 0 | 0 | $1^{ns}$ |
| Absent | 15 (100%) | 15 (100%) | |
| Leaflet damage | | | |
| Present | 0 | 0 | $1^{ns}$ |
| Absent | 15 (100%) | 15 (100%) | |
| Leaflet competency | | | |
| Competent | 14 (100%) | 13 (86.67%) | $1^{ns}$ |
| Incompetent | 1 | 2 (13.33%) | |
| Valve calcification | | | |
| Present | 0 (6.67%) | 0 | $1^{ns}$ |
| Absent | 15 (93.67%) | 15 (100%) | |
| Tissue necrosis | | | |
| Present | 0 | 0 | $1^{ns}$ |
| Absent | 15 (100%) | 15 (100%) | |

**Data was analyzed using Fisher exact test and expressed as frequency
**Figures in parentheses denote corresponding percentage
n= Total number of subjects
ns = non-significant

**Table 3:** Comparison of morphological abnormalities between two study groups after cryopreservation (N=22)

| **Variables** | **Group-A (n1=12)** | **Group-B (n2=10)** | **p-value** |
|---|---|---|---|
| Vegetation | | | |
| Present | 0 | 0 | $1^{ns}$ |
| Absent | 12 (100%) | 10 (100%) | |
| Visible color change | | | |
| Present | 0 | 0 | $1^{ns}$ |
| Absent | 12 (100%) | 10 (100%) | |
| Valve annular diameter shrinkage/dilatation | | | |
| Present | 0 | 0 | $1^{ns}$ |
| Absent | 12 (100%) | 10 (100%) | |
| Leaflet damage | | | |
| Present | 0 | 0 | $1^{ns}$ |

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| | | | |
|---|---|---|---|
| Absent | 12 (100%) | 10 (100%) | |
| Leaflet competency | | | |
| Competent | 0 | 0 | 1 $^{ns}$ |
| Incompetent | 12 (100%) | 10 (100%) | |
| Tissue necrosis | | | |
| Present | 0 | 0 | 1 $^{ns}$ |
| Absent | 12 (100%) | 10 (100%) | |

**Data was analyzed using Fisher exact test and expressed as frequency
**Figures in parentheses denote corresponding percentage
n= Total number of subjects
ns = non-significant

**Table 4:** Comparison of presence of organisms between two study groups before and after antibiotic treatment (N=30)

| **Organism** | **Group-A (n=15)** | **Group-B (n=15)** | **p-value** |
|---|---|---|---|
| Before treatment | | | |
| Present | 2 (13.33) | 7 (46.67) | 0.04$^{s}$ |
| Absent | 13 (86.67) | 8 (53.33) | |
| After treatment | | | |
| Present | 1 (6.67) | 1 (6.67) | 1$^{ns}$ |
| Absent | 14 (93.33) | 14 (93.33) | |

**Table 5:** Comparison of presence of organism between two study groups after cryopreservation (N=22)

| **Organism** | **Group-A (n=12)** | **Group-B (n=10)** | **p-value** |
|---|---|---|---|
| After cryopreservation | | | |
| Present | 0 | 0 | 1$^{ns}$ |
| Absent | 12 (100%) | 10 (100%) | |

## DISCUSSION

This observational study was carried out in the department of Cardiac Surgery at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from July 2016 to June 2018. The study objective was to determine the ability to produce and store clinically usable homograft aortic valve obtained from human cadaver. Total 30 cadaveric hearts were procured and included in this study as per inclusion and exclusion criteria. Hearts were dissected aseptically and aortic homografts were collected. These homografts were allocated equally in two groups depending on the length of homografts. Aortic Sleeves were included in Group-A and Aortic Conduits were included in Group-B. Both groups were treated with modified CLVPA solution at 4 $^{o}$C for 24 hours and then cryopreserved for at least 12 weeks. In terms of demographic characteristics, the mean age in both Group-A and Group-B was found to be remarkably similar, with Group-A averaging 34.27 ± 8.16 years and Group-B averaging 34.20 ± 8.22 years. This aligns with the study

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conducted by Kitamura S et al,[12] and Yankah AC et al,[13] where comparable mean ages were reported in their cohort of valve transplant recipients. Additionally, the gender distribution exhibited no statistically significant difference between the two groups, with Group-A having 11 (73.33%) male and 4 (26.67%) female subjects, and Group-B comprising 12 (80%) male and 3 (20%) female participants. This gender distribution consistency echoes previous studies, contributing to the reliability of the present findings.[12] The examination of BSA values further supported the comparative nature of the study. In Group-A, the mean BSA for males was 1.71 ± 0.17 $m^2$ and for females was 1.55 ± 0.1 $m^2$, while in Group-B, the mean BSA for males and females was 1.69 ± 0.14 $m^2$ and 1.68 ± 0.03 $m^2$, respectively. These values demonstrated no statistically significant difference between the groups, reinforcing the study's emphasis on a balanced and comparable participant cohort. Morphological abnormalities before cryopreservation were minimal in both groups. In Group-A, only 1 (6.67%) valve exhibited leaflet incompetency. In Group-B, 2 (13.33%) valves displayed leaflet incompetency, and 1 (6.67%) valve suffered traumatic damage during dissection. The statistical analysis, however, revealed non-significant differences in the means of morphological abnormalities ($p>0.05$). This resonates with findings from the study by Goffin Y et al,[14] which reported low prevalence rates of morphological abnormalities in cryopreserved valves. After cryopreservation, both groups showed a remarkable preservation of the initially healthy morphologies. None of the valves with apparently healthy morphologies before cryopreservation exhibited any abnormalities post-cryopreservation. This outcome reinforces the efficacy of cryopreservation techniques in maintaining structural integrity, aligning with the findings from O'Brien MF et al,[2] and contributing to the growing body of evidence supporting the reliability of cryopreservation in valve transplantation. Moving to the microbiological outcomes, Group-A and Group-B demonstrated notable differences in the presence of organisms before antibiotic treatment. In Group-A, 2 (13.33%) valves were found contaminated, while Group-B had 7 (46.67%) contaminated valves. This discrepancy was statistically significant ($p<0.05$), reflecting variations in contamination rates between the two groups. These findings resonate with the study by Goffin YA et al,[15] which highlighted differences in contamination rates among different patient groups undergoing valve transplantation. In present study, even though the initial rate of contamination was higher, modified CLPVA solution was more effective (77.78%) in decontamination compared to them. Costa et al.16 analyzed initial 8 years of activities in human heart valve bank at BVCHSC, Brazil. They demonstrated, out of 1491 dissected grafts, 340 (22.8%) were contaminated and it was possible to decontaminate only 259 (76.2%) after adding an antibiotic solution. Reminder 81 grafts (23.8%) showed persistence of organism even after antibiotic treatment. This result is comparable to the study result. Following antibiotic treatment, both groups exhibited successful decontamination, with only 1 (6.67%) valve in each group retaining the organism. This emphasizes the effectiveness of antibiotic intervention in eliminating contaminants. The 3D bar diagram illustrating the effect of antibiotic treatment on contaminated valves

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vividly captures this outcome. Out of the total 9 valves treated with antibiotics, 2 valves retained the organism (1 in each group), while 7 valves were effectively decontaminated (1 in Group-A and 6 in Group-B). These results are consistent with other studies, which emphasized the successful reduction of contaminants through antibiotic intervention.[16,17,18] The microbiological culture findings before antibiotic treatment revealed Pseudomonas aeruginosa as the most prevalent organism, identified in 5 cases, followed by Staphylococcus aureus and Streptococcus epidermidis with 2 cases each. Candida albicans was absent before antibiotic treatment. After antibiotic intervention, Pseudomonas aeruginosa decreased from 5 to 2 cases, while Staphylococcus aureus and Streptococcus epidermidis were completely eradicated. Candida albicans remained absent post-treatment. These numerical values emphasize the specific response of different organisms to antibiotic treatment, aligning with previous studies by Vogt PR et al,[17] and Soo A et al,[19] which identified Pseudomonas aeruginosa as a common contaminant in valve transplantations. The successful eradication of Staphylococcus aureus and Streptococcus epidermidis post-antibiotic treatment mirrors the outcomes reported by Strickett MG et al,[20] showcasing the efficacy of antibiotics against these specific organisms. The effectiveness of cryopreservation in maintaining sterility, where both groups retained sterility (absence of organisms) after cryopreservation. The statistical analysis demonstrated non-significant differences in the means of presence and absence of organisms after cryopreservation between the two groups ($p>0.05$). This aligns with the study by Vogt PR et al,[17] which reported similar success rates in preserving the sterility of cryopreserved valves. The present study, when considered in comparison with existing literature, contributes significantly to the understanding of valve preservation and treatment. The consistent trends observed across multiple studies enhance the credibility of the current findings and underscore the relevance and applicability of the study's outcomes in the broader context of valve transplantation research.

**Limitations of the study:** Because of the time and resource limitation, in spite of utmost effort by the researcher, the study was conducted with small sample size. Hence, it may not be adequate to represent the actual result. Result with larger sample size would be more accurate. The shelf life of the cryopreserved valves were assessed over a limited period. Quality of the valves over longer period should be studied to accurately assess the shelf life. The valves were not assessed microscopically. Both light microscopy and scanning electron microscopy could delineate morphological integrity at cellular and sub-cellular level. Expertise and resources are very limited in this field in our setup. Cell viability and antigenicity of the homograft was not assessed as the resources are very limited at present time.

## CONCLUSIONS

The current study concludes that it is possible and effective to procure aortic homograft from human cadaver for aortic replacement surgery. Microbiological findings reveal significant differences in contamination rates between Aortic Sleeve (AS) and Aortic Conduit (AC) groups, emphasizing the need for tailored interventions. Cryopreservation proves

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effective in maintaining sterility, aligning with established principles. These insights contribute to the broader understanding of successful valve transplantation. This study recommends an institutional continuous study using large numbers of homograft valves should be done to get more comprehensive data on collection, preparation and cryopreservation of homograft valves. This study also recommends future establishment of heart valve bank in this country as well as in the Department of Cardiac Surgery, BSMMU.

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