hepatitis C antibody screening was introduced in May 1990. Despite social screening and serum testing there have been recent reports of HIV and hepatitis C transmission to bone allograft recipients.[23,24] There have been no confirmed reports of hepatitis B virus infection of a bone graft recipient but a case report prior to antibody testing was suggestive.[25] Syphilis infection following allograft surgery has not been reported.[26]

Creutzfeldt-Jacob disease is not routinely tested for in any of the major bone banks having membership with either the American Association of Tissue Banks or the European Association of Tissue Banks. The only valid methodology for exclusion involves histopathological analysis by an accredited histopathologist. By excluding donors with a careful analysis of the clinical history of the donors and relatives for any mental illness (including depression and abnormal behaviour syndromes) and dementia the risks can be minimized. There has not been a documented transmission of Creutzfeldt-Jacob disease with bone. The South Australian bone bank has now issued a disclaimer in relation to Creutzfeldt-Jacob disease with bone that is distributed.

The effectiveness of screening is dependent upon the sensitivity of the antibody test which is reported to be 99.41[27]–99.69%[28] for anti-HIV 1 (Fastek 901, Fujiribio, Japan). The first generation hepatitis C antibody tests were superseded in Australia in 1991 and the third generation antibody tests were introduced mid-1994. Australian and European tissue banks continue to improve screening methods as they are developed and have fortunately avoided the protracted licensing procedures that delayed the introduction of routine HIV screening in the United States and will slow the introduction of hepatitis C antibody screening.[29]

To reduce the chance of missing an infected but sero-negative donor it has recommended that grafts from living donors undergo a quarantine period with a HIV-antibody test 90–180 days following harvesting[5,7] and these recommendations now apply to Australia.[30] By providing a quarantine of 12 or more weeks before testing for the HIV-1 antibody it is estimated that the potential to detect seronegative donors is 99%.[31] A 180 day quarantine and repeat serum test was introduced in 1993.

The addition of a HIV-antigen test remains controversial. Prior to the detection of antibodies it is possible to identify other markers of HIV infection by detection of HIV P24 antigen or by molecular testing using the polymerase chain reaction (PCR). HIV infection has been proven prior to detection of anti-HIV antibodies[32–36] and has been suggested that such molecular technology can be clinically applied to the detection of seronegative at risk individuals[37] or on a larger scale.[38] Applying this technology to allograft donors in the clinical and experimental setting has been disappointing, HIV antigen testing is only in clinical application in Thailand which is an area of extreme risk and only one antigen positive but seronegative donor has been detected by this method.[39]

The p24 antigen assay has been used experimentally to screen large numbers of blood donations including HIV endemic areas but there have been no positive tests from blood which was antibody negative.[40–42] It may be possible to apply the polymerase chain reaction to dried blood spots for diagnostic purposes[37] but at the moment carrying out PCR for diagnosis is only possible in sophisticated reference laboratories and the results must still be viewed with caution.[27,43] These newer technologies are not currently recommended by the American Association of Tissue Banks or Australian blood banks.

Conclusions

The ongoing activities of the South Australian Bone Bank have been described. The bone bank has expanded considerably since its foundation in 1986 and now services a population of 1.4 million people. The demand for allograft bone has risen since the introduction of the bank and is expected to continue for the foreseeable future. This review suggests that bone is the most frequently transplanted non-haematogenous allograft.

The low risk demographics of the donor population combined with an aggressive discard policy have minimized the chance of allograft transmitted disease including bacterial and viral infection. The bank continues to upgrade its pre-donation testing requirements as newer epidemiological information and technology are available. Since the initial report of the bank's activity in its foundation year the donor history has become more stringent and serum testing now includes hepatitis C and improved tests for HIV. A donor quarantine and 180 day retest was introduced in 1993. The estimated probability of HIV infection from this bank is less than 0.2 per million donations (unpubl. data).

The Australian Orthopaedic Association has formed a bone banking sub-committee and is drafting a national standard for bone banks. The national standard is similar to the current practices of the South Australian bank.

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Femoral Head Banking: NUH Tissue Bank Experience

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abstract

National University Hospital Tissue Bank protocol follows guidelines recommended by the American Association of Tissue Banks and the European Association of Tissue Banks using donor selection criteria: medical history, clinical examination, chart review and laboratory tests for acquired immunodeficiency syndrome (AIDS), hepatitis B, hepatitis C, syphilis, and specimen for culture/sensitivity tests. For living donors, repeat testing is performed for AIDS and hepatitis C approximately 180 days after procurement. Femoral heads are procured using the "sterile double jar technique" and stored at −80°C. Our first study of 273 consecutive potential donors undergoing hemiarthroplasty from 1989 to 1994 showed that a high percentage (42.5%) was unsuitable for use. A second study involving 175 potential donors was conducted from 1995 to 2003 after hepatitis C screening was introduced. The bacterial contamination rates in both studies (3.5% and 5.7%) are low. The incidence of other diseases also are low: hepatitis B, 2.3% and syphilis, 1.8% in the first study and hepatitis B, 5.7%; hepatitis C, 0.6%; and syphilis, 5.1% in the second cohort. No cases of AIDS were reported in either study. By 2003, femoral heads were transplanted in 205 patients with a low complication rate of 2.9%.

With increasing public and professional awareness on tissue banking in the past decade, numerous tissue banks have been set up in the Asia-Pacific region following similar guidelines recommended by the American Association of Tissue Banks and the European Association of Tissue Banks.[1] Despite this, hospitals in Singapore and elsewhere in the region continue to procure femoral heads without performing proper donor screening including taking a medical history, conducting a clinical examination of the living donor, and subjecting the donor to the necessary laboratory investigations. Some hospitals store the collected femoral heads in single sterile bottles in electrical freezers at −20°C without proper documentation.

National University Hospital (NUH) autoclaved the femoral heads and stored them in refrigerators (+4°C) for allograft transplantation. This practice ceased when the National University of Singapore Bone Bank was opened in October 1988. These bones are used when bone grafts are needed to fill defects. Such practices must be discouraged, as tissues procured without proper donor screening, procurement and processing procedures are unsuitable for safe tissue transplantation.

Femoral head banking is easier to establish than banks designed to procure tissues from deceased donors.[2,3] Less capital expenditure is involved. The only equipment required is electrical freezers for storage of bones at −80°C. There is no need to acquire other equipment including a band saw, water bath, lyophilizer, laminar flow cabinet and vacuum sealer if only deep-frozen bones are processed. A femoral head bank also does not require a complex manpower organization, large physical infrastructure, and substantial logistic support compared to a bank designed for procuring tissues from deceased donors.[4,5]

This article describes the femoral head banking program conducted by National University Hospital Tissue Bank since its establishment in October 1988. It also describes our study from 1988 to 1994 on

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donor selection criteria to determine the proportion of femoral heads from potential living donors that were suitable for bone transplantation. A second study from 1995 to 2003 and the introduction of laboratory testing for hepatitis C in January 1995 reported on the results of laboratory testing for procured femoral heads. Finally, the clinical application of the femoral heads banked also is described including the complication rate.

MATERIALS AND METHODS

The NUH Tissue Bank was established in October 1988 as a research tissue bank with a research grant from the National University of Singapore (NUS). It was previously known as NUS Bone Bank, with two electrical freezers for storing bones at −80°C.[6,7]

For the first three years, in addition to conducting basic research, its clinical activity consisted of procuring bones from living donors,[6,7] mostly femoral heads from elderly patients undergoing hemiarthroplasty for displaced femoral neck fractures.

Up to June 30, 2003, 449 bones were procured from 440 living donors. This included 297 femoral heads from elderly patients aged >60 years undergoing hemiarthroplasty for displaced femoral neck fracture and 138 total knee replacement (TKR) bone slices from elderly patients undergoing TKR for osteoarthritis of the knee. Other bones procured included three humeral heads, one radial head, one rib, three tibia, three fibula, three lower end femur, and three patellae. The patellae were procured from three living donors undergoing above knee amputation for lower-limb ischemia without sepsis.

In addition, since the first deceased donor procurement in November 1991, NUH Tissue Bank also has pursued a successful Deceased Donor Program. Up to June 2003, 1055 bones and soft-tissue specimens were procured from 63 deceased donors.

This article focuses only on the femoral head banking program conducted by the NUH Tissue Bank.

Bone banking and transplantation is legal in Singapore under the Medical (Therapy Education and Research) Act of 1972, allowing procurement only if donors or relatives or next of kin have given consent.[6,7]

The NUH Tissue Bank Protocol follows similar guidelines for banking of musculoskeletal tissues recommended by the American Association of Tissue Banks and the European Association of Tissue Banks.[1,6]

Strict donor selection criteria is the most important step for safe tissue transplantation practice.[8,9] All potential living donors must be screened to exclude AIDS, active systemic infection, active infection of bone procured, malignancy, active hepatitis or unexplained jaundice, diffuse connective tissue disease, metabolic bone disease, death from unknown cause, chronic drug abuse, heavy irradiation of bone procured, exposure to toxic substances, patients on a respirator for >72 hours, and patients on steroids for >5-7 days.[4,8,10,11]

Potential donors testing positive for AIDS, hepatitis B, hepatitis C, syphilis or bacterial contamination are excluded from the femoral head banking program.

Following a detailed medical history, the donors undergo a thorough clinical examination. In addition, a chart review is performed to exclude any fever. Laboratory investigations then are performed to test for AIDS (Anti-HIV1, Anti-HIV2), hepatitis B (HbsAg), hepatitis C (Anti-HCV) and syphilis (RPR, followed by TPHA if positive). A small piece of bone also is sent for culture and sensitivity tests for aerobic and anaerobic organisms.[3,4,12,13] For living donors, repeat testing for AIDS (Anti-HIV1 and Anti-HIV2) and hepatitis C (HCV) is performed at least 180 days following the donation.

Informed consent first must be obtained from the potential living donor or next of kin before the femoral head can be procured, including consent to undergo the necessary laboratory testing.[13] The latter is important because by law, a positive HIV test must be disclosed to the patient and to the Ministry of Health in Singapore. Patients also are informed that the cost of all laboratory testing is covered by the tissue bank. Potential donors testing positive for AIDS, hepatitis B, hepatitis C, syphilis or bacterial contamination are excluded from the femoral head banking program and their femoral heads are discarded.[2,12]

The bank maintains accurate and detailed documentation. Documentation is performed using a Living Donor Form and a Consent Form for Living Donors. For each living donor, the NUH Tissue Bank uses a separate NUH Tissue Bank Medical Record Folder (color coded red) specific for that donor. All forms for this patient including the results of the laboratory tests performed for this donor are kept in the folder. Such a meticulous documentation system is essential for maintaining a good quality control system of the tissue bank and also facilitates audits to be conducted readily.[4,14] In addition, entries also are made in a Living Donor Log Book and are recorded on a computer.

Suitable living donors for femoral head banking include all elderly patients aged ≥60 years who have sustained a displaced femoral neck fracture—Garden's Grade III or Grade IV—and who are scheduled to undergo a hemiarthroplasty (with either a Moore's or a Thompson's prosthesis).[15]

During hemiarthroplasty, the femoral head removed under sterile conditions[8,9,12,16] first is measured with a caliper to determine the diameter of the Moore's or Thompson's prosthesis needed to replace the femoral head. The bone then is flushed repeatedly with saline to remove all blood and debris until the returns are clear.

A small piece of bone is removed from the femoral head with a rongeur and is

Table 1
Medical Contraindications

Contraindication	No. Patients
Malignancy	19
Tuberculosis	16
Chest infection	10
Urinary tract infection	14
Bed sores	5
Infected foot ulcers	2
Lower limb infections	4
Fever	2
Renal failure	7
Rheumatoid arthritis	5
Thyroid disease	4
Systemic lupus erythematosis	3
Degenerative neurological disorders	4
Pathological fracture	2
Long-term steroids	2
Avascular necrosis	3
Liver disease	1
Total	103

placed in a sterile container to be sent for culture and sensitivity tests for aerobic and anaerobic organisms. The head then is soaked in 200 mL of normal saline containing 250 mg ampicillin and 250 mg cloxacillin for approximately 30 minutes. It then is inserted into an autoclaved, sterile, inner jar with a screw-on lid that is then closed. The inner jar then is placed into a slightly larger, autoclaved, sterile, outer glass jar with an air seal lock over a rubber bung. The "sterile double jar" then is stored in an electrical freezer at −80°C in the NUH Tissue Bank.

When received by the bank, the technician stores the sterile double jar initially in a "quarantine freezer" until the results of all laboratory tests are processed. If any results are positive, the femoral head must be discarded. If all the results are negative, the femoral head is suitable for use and then is transferred from the "quarantine freezer" to the "ready for use freezer."

Table 2
Positive Laboratory Tests

Screening Test	No. Patients
Positive culture	6
Hepatitis B	4
Syphilis	3
Total	13

Deep-freezing must be maintained at −80°C in both freezers. This can be verified by recording the thermograph attached to the freezer. Both freezers must run on a "red socket switch" with a back-up generator to provide emergency electrical power in case of electrical failure. In our protocol, no femoral head is released for transplantation until the deep-freezing process has been conducted for at least 4 weeks to ensure that the immunogenicity of the femoral head has been reduced to a minimum.

Results

For this study, 273 consecutive potential living donors aged ≥60 years, who had sustained a displaced femoral neck fracture (Garden's Grade III or Grade IV) undergoing hemiarthroplasty (using Moore's or Thompson's Prosthesis) at National University Hospital from September 1989 to December 1994 were analyzed.

Average donor age was 67.5 years (range: 60-83 years). There were 210 women and 63 men. All patients were subjected to detailed medical history, a thorough clinical examination, as well as a chart review to rule out medical contraindications.

Table 1 shows that medical contraindications were present in 103 (37.7%) patients. These patients were rejected from donating their bones. The most common contraindications included malignancy, tuberculosis, chest infection, and urinary tract infection. The remaining 170 potential donors then were subjected to laboratory testing to rule out AIDS (Anti-HIV1, Anti-HIV2), hepatitis B (HbsAg), and syphilis (RPR, followed by TPHA if positive). Hepatitis C testing (Anti-HCV) was not available during this period. Table 2 shows that of the remaining 170 donors subjected to laboratory testing, 13 (7.6%) donors had positive laboratory test results.

No donors tested positive for AIDS even after the repeat testing. Six (3.5%) patients had bacterial contamination (Table 3), the majority due to contamination from skin commensals (*Staphylococcus epidermidis* and *S aureus*). Hepatitis B was found in 4 (2.3%) donors and syphilis in 3 (1.8%). This study showed that of 273 potential living donors, 157 (57.5%) were found using strict donor selection criteria and laboratory testing to be safe and suitable for femoral head banking and transplantation.

Because laboratory testing for hepatitis C (Anti-HCV) only was conducted by the NUH Tissue Bank from January 1995 onwards, a second study was performed for all living donors (175) who were found to not have any medical contraindication and then subjected to laboratory tests from January 1995 to June 2003. The results of positive laboratory tests were analyzed. Average donor age was 68.5 (range: 60-97 years). There were 133 women and 42 men.

All 4 cases of pseudoarthrosis with implant failure required revision of the fusion anteriorly using a titanium cage filled with autografts.

Table 4 shows that of 175 femoral heads, 35 (20%) were discarded, 5 (2.8%) due to incomplete laboratory testing and 30 (17.1%) due to positive results. Ten (5.7%) patients showed bacterial contamination. Ten (5.7%) were positive for hepatitis B. One patient was positive for hepatitis C (0.6%). No additional positive

result for hepatitis C was found with repeat testing. Nine (5.1%) patients tested positive for syphilis. None of the patients tested positive for AIDS even after repeat testing. Of the 10 patients with bacterial contamination, the majority were due to skin commensals (*S aureus* and *Corynebacterium* species) (Table 5).

Up to June 30, 2003, femoral head transplantations have been performed in 205 patients, including 95 for spine surgery, 31 for curettage and bone grafting for malignant and benign bone lesions, 30 for trauma, 21 for hip surgery, and 9 for knee surgery (Table 6).

Complications occurred in 6 (2.9%) of 205 transplantations. Of 93 posterolateral fusions, 6 (6.5%) complications occurred: 1 due to deep infection, 1 due to superficial infection, and 4 due to pseudoarthrosis with implant failure. The deep infection resolved with extensive debridement, removal of all grafts, and antibiotics. The superficial infection resolved with debridement, daily dressings, and antibiotics. All 4 cases of pseudoarthrosis with implant failure required revision of the fusion anteriorly using a titanium cage filled with autografts.

Table 3

Bacterial Contamination

Organism	No. Patients
*Staphylococcus epidermidis**	3
Staphylococcus aureus	2
Bacterioides fragilis	1
Total	6

**On enrichment.*

Table 4

Positive Laboratory Tests*

Screening Test	No. Patients
Positive culture	10
Hepatitis B	10
Hepatitis C	1
Syphilis	9
Incompfete testing	5
Total	35

**Second study.*

Table 5

Bacterial Contamination*

Organism	No. Patients
Coagulase negative	
Staphylococcus aureus†	5
Staphylococcus epidermis†	1
Corynebacterium species	1
Pseudomonas stutzeri†	1
Acinetobacter baumanii†	1
Alcaligenes faecalis†	1

**Second study.*
†*On enrichment.*

Table 6

Femoral Head Transplantation Performed

Indication	No. Cases
Spine	
Posterior spinal fusion	93
Anterior spinal fusion	2
Hip	
Revision THR	18
Primary THR	2
Hip arthrodesis	1
Knee	
Revision TKR	7
Knee arthrodesis	2
Ankle/foot	
Subtalar fusion	3
Triple arthrodesis	1
Trauma	
Calcaneum fracture	9
Tibial condyle fracture	9
Lower-end radial fracture	2
Intertrochanteric femoral fracture	1
Nonunion reconstruction	9
Currettage and bone grafting	
Giant-cell tumor	5
Chondroblastoma	5
Fibrous dysplasia	10
Simple bone cyst	6
Aneurysmal bone cyst	5
Other bone lesions*	15
Total	205

Abbreviations: THR=total hip replacement and TKR=total knee replacement.
**Including Maxillo facial lesions.*

DISCUSSION

The fact that some hospitals collect femoral heads without proper screening, laboratory testing and proper storage conditions for use in orthopedic surgery is proof that allografts are required in orthopedic practice. Nevertheless, such a practice must be discouraged to minimize the risk of transmitting disease from donor to recipient. Bone banking must be confined to banks with the facilities, resources, and manpower to procure and process bone tissues following guidelines similar to those recommended by the American Association of Tissue Banks and the European Association of Tissue Banks. This is important since disease transmission has occurred with bone transplantation.[17,18]

We advocate storing the femoral head using the "sterile double glass jar technique," as such containers save storage space in the electrical freezers allowing more specimens to be stored and easy handling of specimens. Tomford et al[2] advocated storing such femoral heads in an inner sterile specimen jar with a screw-on lid wrapped in three sterile hand towels and a sterile plastic bag. Saies and Davidson[3] used an inner sterile plastic bag that was sealed and then placed in an outer sterile plastic specimen container.

Our first study conducted from 1989 to 1994 showed that a high proportion–approximately 42.5%–of potential femoral head donors were unsuitable for use and were discarded. This finding showed the

What is already known on this topic

Femoral head banking, donor selection criteria, and the bacterial contamination rate with femoral head banking have been previously reported.

What this article adds

This article describes a new "sterile double jar technique" for storing procured femoral heads.

This is the first article that reports on the incidence of hepatitis C, hepatitis E, and syphilis in potential living bone donors.

importance of conducting proper donor selection criteria by detailed medical history, thorough clinical examination, chart review, and laboratory tests. The practice by some hospitals of procuring femoral heads without conducting any screening must be discouraged as unsafe for bone allograft transplantation. It is interesting to note that Saies and Davidson[3] also reported 46 (46%) of 100 femoral heads to be unsafe for banking—a figure similar to our finding.

The rate of bacterial contamination was 3.5% in our first study and 5.7% in our second cohort. Tomford et al[2] found a similar low bacterial contamination rate of 2.6% and Salmela et al[16] a rate of 5.2%. The bacterial contamination rate was high (17%) in the study by Saies and Davidson[3] and 22% in the study by Sommerville et al.[19]

The incidence of positive results for other diseases screened by laboratory tests were low in both of our studies: 2.3% for hepatitis B and 1.8% for syphilis in our first study and 5.7% for hepatitis B, 0.6 % for hepatitis C, and 5.1% for syphilis in our second cohort. In contrast, Saies and Davidson[3] reported zero incidence of hepatitis A or B (86 tested) and syphilis (86 tested).

It is encouraging to note that no positive results for AIDS were reported in either of our studies. Saies and Davidson[3] also reported no incidence (81 tested) of AIDS. This zero incidence for AIDS would indicate that femoral heads are the safest bone allografts to use because they belong to an elderly population group with low risk for HIV transmission compared to the younger age group associated with deceased donors.

The complication rate of femoral head allograft transplantation in our series also is low, 6 (2.9%) of 205 recipients.

Conclusion

Femoral head banking from elderly living donors is the safest type of bone to procure for transplantation compared to long bones from younger deceased donors. In addition, proper meticulous donor selection criteria must be performed and the bones must be procured sterile and stored in freezers at −80°C using the sterile double jar technique. ▣

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Infections after bone allograft surgery: a prospective study by a hospital bone bank using frozen femoral heads from living donors

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Abstract In the advent of the EU guidelines 2004/23/EG and 2006/17/EG requiring extensive safety and quality steps in bone banking, the prevalence and risk of infection disease transmission from bone allograft needs to be reconsidered. Therefore, we prospectively reviewed the screening process of bone donations and the outcome of surgeries utilizing bone allografts from our internal hospital bone bank with regard to infections according to CDC criteria. One-hundred and eighty-eight allogenic bone transplantation procedures in 160 patients were followed-up for 12–64 months (mean 32 months). Bacterial infection occurred in 11 patients, the overall infection rate therefore was 6.9%. After review of the clinical and intraoperative findings, none of the infections were likely to have been caused by the bone graft. Although no follow-up serologic testing was performed, no HIV of hepatitis infections were observed. Frozen bone allografts derived from live donors and provided by hospitals can generally be considered safe. However, without new and relevant clinical expertise, continuing this technique will be impeded by the new EU guidelines and their national implementations.

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Keywords Bone banking · Allograft transplantation · Infection transmission · Allogenic bone

Introduction

In Europe, and especially in Germany, the most widely distributed method of supplying allogenic bone grafts is internal hospital bone banks (Aho et al. 1998; Hart et al. 1986; Ivory and Thomas 1993; Meermans et al. 2007; Nather and David 2007; Schreurs et al. 2003; Sutherland et al. 1997). Femoral heads retrieved during primary hip arthroplasty procedures are usually stored in a fresh-frozen fashion. Many institutions do not perform additional preparation steps, such as gamma irradiation or heat (Pruss et al. 2003). Although the use of bone allografts has grown, optimal use has been restricted by availability and concerns for recipient safety (Tomford 2000). Common complications of allogenic bone transplantation include non-union (Mankin et al. 1996), viral disease transmission (CDC 1988; Conrad et al. 1995), and bacterial infection (Aho et al. 1998; Chapman and Villar 1992; Chiu et al. 2004; Deijkers et al. 1997, Hou et al. 2005; Liu et al. 2002; Mankin et al. 1983; Nather and David 2007; Sommerville et al. 2000; Sutherland et al. 1997; Tomford et al. 1981, 1990; Winter et al. 2005).

Recently, the EU released guidelines 2004/23/EG and 2006/17/EG (EU 2004) which redefine the framework for human tissue transplantation including

bone allograft transplantation. While these new directives do not alter the requested measures to prevent disease transmission by donor selection and bone graft procession, the administrative burden has increased (Pruss et al. 2005) [§§ 20b,c AMG (Approval for Procurement, Testing and Processing resp. under the responsibility of the local pharmaceutical authorities), § 21a AMG (License for Tissue preparations under responsibility of the Paul-Ehrlich-Institute)]. A "pharmaceutical-manufacturer-license" now has to be applied by the German health authority in order to maintain an already established non-commercial "fresh-frozen" bone bank (Pruss et al. 2005). In consequence, many hospitals had to consider discontinuing their internal hospital bone banks (Pruss and von Versen 2007).

With regard to these inconveniences and the well known risk of induction and transmission of diseases by bone transplantation, we feel that bone banks have to be analyzed routinely—both in terms of cost effectiveness and clinical safety (Floren et al. 2007). Therefore, we prospectively analyzed our hospital's live donor bone bank experience and investigated the nature and frequency of infections in patients receiving bone allografts.

Methods

The bone banking process

The internal bone bank of our department was established in the late 1980s. We rely on femoral heads obtained during primary total hip replacement exclusively. Bone donation is voluntary and no financial or other benefits are granted. The allografts are solely utilized to meet our own demands and not distributed commercially.

Donors are selected by a thorough standardized patient history (Bundesärztekammer 2001) enquiring about former infections, travel to countries with epidemic diseases, tumor diseases, behaviors with increased risk for infectious diseases, etc. Routine preoperative serological testing includes glutamyl pyruvic transaminase (GPT), hepatitis B (HBs-antigen and anti-HBc-antibodies), hepatitis C (HCV-antibodies and HCV-RNA), syphilis (*Treponema pallidum* hemagglutination titre), and HIV (HIV1/2-antibodies). Donors are retested at 6 months for anti-HIV, HBs-antigen, anti-HBc-antibodies and anti-HCV. Bone donation is declined if the prospective donor has a positive infectious disease test or meets any of the other exclusion criteria.

During graft retrieval, a swab culture is taken from every donation. The swabs are cultured aerobically and anaerobically on blood, MacConkey, chocolate and CIN (Cefsulodin, Irgasan, Novobiocin) agar plates as well as in thioglycolate broth at 37°C for 48 h before evaluation. After removing all soft tissues and cartilage, the femoral head grafts are placed into a sterile polypropylene container which is itself placed into a second sterile container. Bone grafts are then frozen in a refrigerator at −70°C (THG 040, Thalheimer, Ellwangen, Germany).

Bone grafts are discarded from the bone bank if (1) the culture is positive, (2) donors show any clinical sign of infection in the postoperative course or if (3) the serological retest (for Hepatitis and HIV, 6 months postoperatively) indicates infection or (4) patients refuse serological retesting. We do not perform routine irradiation nor do we irradiate contaminated bone grafts. In addition, bone grafts are not incubated in solution containing antibiotics.

Perioperative antibiotic prophylaxis

In primary total hip replacement we routinely perform antibiotic prophylaxis with a second-generation cephalosporin (cefuroxime) given as a preoperative intravenous short infusion ("single shot"). Cephalosporin prophylaxis is given during hip revision surgery to bone allograft recipients before allograft transplantation.

Research method

We registered all consecutive donations during a 30 month period and recorded all reasons for rejecting prospective donors and rejecting donations. In case of a declined bone donation, we noted the reason for rejection. We prospectively analyzed the clinical indications and postoperative course of all patients who received allografts during surgery (the index operation). Patient records were analyzed according to the centers for disease control (CDC) criteria for organ surgical site infection (Table 1; Horan et al. 1992). Organ surgical site in this paper is synonymous to site of allograft implantation (e.g., hip or

Table 1 CDC definition of organ surgical site infection (SSI)

Organ SSI must meet the following criteria: (Horan et al. 1992)

1. The infection occurs within 30 days after the operative procedure if no implant is in place or within 1 year if implant is in place
2. The infection appears to be related to the operative procedure
3. The infection involves any part of the anatomy, other than the incision, opened or manipulated during the operative procedure

In addition, one of the following

a. Purulent drainage from a drain that is placed through a stab wound into the organ
b. Organisms isolated from an aseptically obtained culture or fluid or tissue in the organ
c. An abscess or other evidence of infection involving the organ that is found on direct examination, during reoperation, or by histopathologic or radiologic examination
d. Diagnosis of an organ SSI by surgeon or attending physician

knee joint). In case of infection, the identified bacteria (if possible) and the clinical consequences were registered. According to CDC criteria, we chose 12 months as the minimum duration of follow-up.

Results

Rejected bone donations

Over the period of 30 months, 629 primary hip replacements were performed. Three hundred and seventy-eight potential donations were declined in total. Of these, only one donation was rejected due to a positive swab culture (Coagulase-negative *Staphylococcus*) while 23 femoral heads were discarded due to possible contamination during preparation (rejected without swab culture). Serologic testing led to rejection on 64 occasions (anti-HBc in 41, anti-HCV in 5, TPHA in 6, elevation of glutamyl pyruvic transferase in 12, no case of anti-HIV or HCV-RNA) while a missing serological retest 6 months after donation led to rejection in 29 cases. 13 patients reported about hepatitis in their history. The remaining 248 potential bone donations were rejected due to other reasons, e.g. autologous transplantation, poor bone quality, tumor or rheumatic diseases, suspicious patient history for infections, etc.

Recipient follow-up

In total 251 allogenic bone transplantations were performed in 220 patients. One-hundred-sixty patients receiving 188 allografts could be followed-up for a minimum of 12-months. Overall, 60 patients (63 allografts) had a follow-up of less than 12 months. They were not included in the statistical analysis, even though during the available average follow-up of 4 ± 3.3 months no infection according to CDC criteria had occurred. Six of these patients (seven allografts) died due to conditions not related to the index procedure.

Therefore, only 160 patients with 188 allografts were included into the statistical analysis with a minimum follow-up of 12 months [mean 32 months ± 13.4 (range 12–64 months)]. The mean age at the time of index surgery was 59 ± 15.1 years (11–83 years). The majority of bone allografts were used in hip revision arthroplasty and lumbar interbody fusions (Table 2).

Table 2 Bone allograft usage and indications for surgery

Indications for bone allograft usage	Total amount	Infections
One-stage THA revision (cup and stem)	35	4
Re-implantation in two-stage THA revision	9	3
THA revision: cup	43	1
THA revision: stem	9	0
TKA revision	2	0
Posterior-lateral interbody fusion	6	1
Anterior lumbar interbody fusion	31	0
Cancellous bone inserted into cage at lumbar spine	17	0
Cancellous bone inserted into cage at thoracic spine	13	0
Cyst filling	1	0
Tumor	2	0
Revision of pseudarthrosis	5	0
Osteotomy	5	2
Arthrodesis	4	0
Fracture	5	0
Defect after removal of metal hardware	1	0
Total	188	11

THA total hip arthroplasty, *TKA* total knee arthroplasty

Analysis for infection

There were 160 patients (188 allografts) available with a minimum follow up of 12 months. We registered 11 cases of infection overall [mean duration until infection 4.35 ± 10 months (0–38, 5 months)]. Therefore, the overall infection rate of operations reviewed in this article was 6.9% (11/160 cases). If only cases of infection confirmed by positive cultures are taken into consideration, the infection rate drops to 5.0% (8/160 cases).

The majority of infections occurred after revision arthroplasty procedures: four after one stage total hip revision arthroplasty presumed aseptic, one after one stage revision of an acetabular component presumed aseptic, three following re-implantation of a hip prosthesis preceded by septic hip prosthesis removal and temporary joint spacer interposition (septic two-stage revision), two after osteotomies for alignment correction, and one after posterior cervical fusion (Table 3).

Careful analysis of patient records revealed that an association between bone allograft procedure and infection could be conscientiously denied in the majority of these cases (Table 3). In three cases (3, 8, 9) the index operation actually was the re-implantation procedure of a septic two-stage hip arthroplasty revision. In all three cases, the overall treatment failed as infections reoccurred after the index operation.

In cases 4 and 5, bacterial growth in the routinely taken intraoperative swabs surprisingly demonstrated bacterial growth in both cases previously classified aseptic. The probes were taken during the index operation prior to allograft implantation, excluding an association with the graft though.

In case no. 2, a superficial wound infection occurred after the index procedure. The local defect could not be controlled followed by a subsequent deep infection in the later postoperative course.

In case 1 the indication for the index operation was suspected allergy to biocement components at the site of a total knee arthroplasty (TKA). The patient primarily complained about dull pain and chronic effusion in the affected TKA and symptoms persisted after the one stage exchange. In a histological specimen finally investigated, a chronic inflammatory infiltrate lead to the assumption of a chronic low-grade infection treated by two-stage revision arthroplasty and a course of antibiotics. There obviously was no association to the allograft.

The indication to the index operation in case 10, a femoral corrective osteotomy, was angular deviation following a grade 3 open fracture with multiple soft tissue revision procedures preceding the index operation.

Finally, in the three remaining cases (No. 6, 7, 11) an infection transmitted or induced by the allograft cannot be completely excluded. On the other hand, none of these infections can be clearly attributed to the allograft. In case 6, bacteria were proven at a different location of the situs, while a swab from the site of the allograft remained sterile. Infection closely followed a subsequent revision operation due to a pure mechanical problem (recurring dislocation) in case 7, while the infection in case 11 occurred in a quadriplegic patient who had a sacral ulcer debridement before, with partially the same bacteria proven in the ulcer as in the infection following the index operation (cervical fusion).

One additional infection occurred 3 years after presumed aseptic revision knee arthroplasty. After uneventful three postoperative years, the patient sustained infection. The case has to be considered as late, most likely haematogenic according to Segawa et al. (1999) and does not meet CDC criteria. It was not respected in our analysis.

We observed no infections that were proven or likely to have been caused by a contaminated allograft. In three cases, infection due to a contaminated allograft can not be ruled out sufficiently to leave it out of consideration. Therefore, the estimated maximal rate for infections possibly associated with the allograft is 1.9% (3/160 cases).

Discussion

Recently, the European Council and the European Parliament have redefined the directives for allogenic bone transplantation. Their national implementation by law resulted in increased organizational requirements and a new authorisation process even for long-time established bone banks. Although the rules for obtaining and processing allografts remained unchanged, many hospital internal bone banks are at risk of not receiving permission to continue. In Germany, they are now no longer able to operate

Table 3 Characteristics of infection cases after allogenic bone transplantation

No.	Age	Surgery	Bacteria	Clinical findings
1	70	TKA one-stage revision	None proven	Suspected allergy before index operation, chronic inflammatory infiltrate on histological examination and identical complaints before and after index operation lead to diagnosis of chronic low grade infection
2	52	THA one-stage revision	None proven	Skin necrosis at greater trochanter, followed by greater trochanter lysis and fistula
3	67	THA septic two-stage revision	Coagulase-negative *Staphylococcus*	Four soft tissue revisions after THR one-stage revision preceding index operation
4	71	THA one-stage revision	*Staphylococcus epidermidis*	Unsuspected positive swab culture taken from joint before allograft implantation. The index operation was followed by multiple revisions, ending in Girdlestone situation
5	42	Humeral corrective osteotomy	*Enterobacter cloacae*	Persistent infection/low grade osteomyelitis after proximal humerus fracture, ORIF, and deep wound infection 6 months before. Unsuspected positive intraoperative swab culture taken from bone bed before allograft implantation
6	62	THA cup revision	None proven	During a subsequent revision 1 month later, purulence was found in the joint, but bone allograft underneath cup was unsuspicious
7	52	THA one-stage revision	*Enterococcus faecalis*	No clinical signs of infection after index procedure. Early infection after subsequent revision due to recurring dislocations 3 months after index operation
8	52	THA septic two-stage revision	*Staphylococcus aureus*	Sterile swab culture taken from site of allograft (acetabulum) during revision, infection found around the femur, classified as persistent infection
9	64	THA septic two-stage revision	*Enterococcus faecalis*	*Enterococcus faecalis* identified before and after index operation—persistent infection
10	30	Femoral corrective osteotomy	*Enterococcus faecalis*	Persistent infection/low grade osteomyelitis after distal femur fracture with large skin defects and compartment syndrome—multiple revisions 1 year before index operation. Definite infection before index operation, *E. faecalis* not proven before though
11	55	Posterior-lateral cervical fusion	*Enterococcus faecalis*, *Corynebacterium xerosis*	Pt. with spinal cord injury and treatment for an infected sacral ulcer 4 weeks prior to index operation, identical bacteria cervical and sacral

ORIF open reduction and internal fixation, *THA* total hip arthroplasty, *TKA* total knee arthroplasty

outside the national drug law (Pruss and von Versen 2007). By analysing our internal hospital bone bank, we intended to contribute to the discussion between orthopaedic surgeons who are in doubt if continuing their own bone banks is useful.

Infections continue to be one of the major reasons for concern when allograft bone is used (Tomford 2000). Previous reports regarding internal hospital bone banks have demonstrated large differences in infection rates associated with bone allograft procedures (Table 4). The 6.9% rate of postoperative infection rate observed in our study is consistent with rates of 0–12.2% for serious or deep postoperative infections reported by others (Table 4). However, methods of bone allograft obtainment and processing differ as well as duration of follow up in these reports. Also, infection rates are certainly known to differ according to the definition for infection (Chiew and Theis 2007).

Our study shows that contaminated allografts do not play a major role in the infections commonly observed after orthopaedic surgical procedures. There

Table 4 Infection rates following use of femoral head allografts obtained from living donors during hip arthroplasty

Author	Follow-up	Infections overall (deep)/transplantations	Infection rate (%)
Hart et al. (1986)	24 months	0/101	0
Meermans (2007)	36 months	0/94	0
Nather and David (2007)	Not reported	2(1)/205	0.9(0.05)
Hou et al. (2005)	Not reported	17/1353	1.3
Halliday (2003)	60 months	5/226	2.2
Chiu et al. (2004)	2–72 months	3(1)/81	3.2(1)
Aho et al. (1998)[a]	Not reported	2	3.4
Schreurs et al. (2003)	36 months	2/35	5.7
Ivory and Thomas (1993)	Not reported	7(2)/59	11.9(3.4)
Sutherland et al. (1997)[b]	Not reported	10(5)/82	12.2(6)

[a] THA revision only

[b] Separation between minor and major infections—major infections/infection rates of major cases are shown in brackets

were no cases in whom the allograft was concluded to be the cause of the infection. In the majority of infections proven by culture, the identified bacteria had already been present before bone graft implantation. In these cases, the index operation was either part of the overall treatment for infection, as e.g. in two-stage septic revision arthroplasty, or the identification of bacteria came unsuspected. We agree with others that preoperative joint aspirates and cultures can be helpful in detecting clinically inapparent infection (Segawa et al. 1999). In a retrospective review of 324 mainly massive structural allografts Tomford et al. (1990) found 21 infections. Using a patient history workup similar to our method, they concluded that in suspicion for the allograft to play a causal role for infection was to be raised in a single occasion.

In summary, our results confirm the safety of allograft femoral head transplantation from living donors via an internal hospital bone bank. We recommend obtaining a "pharmaceutical-manufacturer-license" for hospitals with a large enough surgical volume to allow running an internal hospital bone bank.

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Bioburden assessment of banked bone used for allografts

Kerry Varettas · Peter Taylor

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Abstract Allograft bone is commonly used in reconstructive orthopaedic surgery and needs to be assessed for bioburden before transplant. The Microbiology Department of the South Eastern Area Laboratory Services (SEALS), located at the St. George Hospital, Sydney, has provided this service to the New South Wales (NSW) Bone Bank. This study reviewed the organisms isolated from femoral head allografts of living donors from the NSW Bone Bank over a 7-year period. It was found that growth was reported from 4.9% of samples with the predominant organism being coagulase-negative staphylococci. This review will focus on the micro-organisms isolated, the interaction of the laboratory with the bone bank, the relevance of the bioburden assessment in the overall quality process and patient safety.

Keywords Allograft · Bioburden · Bone bank · Contamination

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Introduction

This laboratory has been involved with the bioburden assessment of allograft bone samples used in reconstructive orthopaedic surgery for more than 10 years. During this period this laboratory has been audited by the Therapeutic Goods Administration (TGA) for services provided to bone banks.

Bone banks are non-profit organisations that co-ordinate the collection, screening, storage and distribution of donated human bone for transplant. Allograft bone has been determined to be a therapeutic good under the Therapeutic Goods Act 1989. This Act requires all bone banks to be licensed by TGA and comply with the principles and procedures of the Code of Good Manufacturing Practice (GMP)—Human Blood and Tissues (August 2000) ensuring standards of quality are maintained throughout the manufacture of the bone. These strictly controlled processes are enforced through a system of mandatory audits. The Code of GMP also requires that mandatory screening tests for donor suitability be performed by a TGA licensed laboratory.

In addition to testing of each donor's blood for blood borne infectious diseases, the microbial bioburden of bone samples is also assessed. Other studies (Sommerville et al 2000, Vehmeyer et al 2002, Journeaux et al 1999, Judas et al 2005, Chapman and Villar 1992) revealed a range of contamination rates from 13 to 22%. A semi-quantitative bioburden assessment is performed on bone surfaces but for bone

fragments only qualitative assessment culture is possible. Donated bone is then sterilised by gamma irradiation before being released for use. The presence of an exceptionally high bacterial count, or the isolation of an organism considered as clinically significant, may influence the decision to release bone even though that sample had been irradiated.

Allograft bone is commonly obtained through the donation of femoral heads removed during hip replacement surgery which is performed at remote sites, placing additional demands on the quality system for bone transport leading to storage, processing and testing.

This review will focus on the micro-organisms isolated from femoral head bone retrieved from living donors during surgery and sent directly to the New South Wales (NSW) Bone Bank. The interaction of the laboratory with the bone bank, the relevance of the bioburden assessment in the overall quality process and patient safety will be discussed.

Method

Bone samples

Samples were collected from surgically removed femoral heads, by operating theatre staff trained by the NSW Bone Bank. These were received in the laboratory as a set of 2 or 3 samples per patient episode. Each set consisted of a bone swab and a bone chip and/or piece of femoral head capsule. Bone chips and capsules were analysed as one group of bone fragments because both samples were not always received. Where patients had a bilateral total hip replacement, a set of left and right hip samples were collected and each set was counted as a separate episode.

Media

Bilayered blood agar (HBA, Columbia agar, Oxoid CM 331 with 7% defibrinated horse blood on Columbia agar base Oxoid CM 331).

Chocolate agar (CHOC, Columbia agar base, Oxoid CM 331, with 9% defibrinated horse blood heated at 75°C for 5 min).

HBA and CHOC were made in the SEALS Media Production Unit, Sydney, NSW.

Cooked meat media (CMM, TM0102) was purchased from Oxoid (Basingstoke, Hampshire, UK).

Inoculation of sample

All samples and media were handled and inoculated in a Class II biological safety cabinet.

The swabs were inoculated onto CHOC and HBA then placed in CMM. Bone fragments were each inoculated directly into CMM.

The agar plates were incubated for a total period of 5 days. The CMM was sub-cultured after 48 h incubation onto CHOC and HBA which were then incubated for 3 days. The CMM was re-incubated for a total of 12 days, after which the clarity of the broth was examined. If visibly clear, the CMM was discarded; if turbid, it was subcultured onto CHOC and HBA and incubated for 48 h.

Incubation of media

HBA was incubated anaerobically, CHOC in 8% CO_2, CMM aerobically, at 35°C.

Culture interpretation

Growth from swab cultures were arbitrarily recorded as scanty (1–10 colonies/plate), light (11–100 colonies/plate), moderate (101–1,000 colonies/plate), heavy (>1,000 colonies/plate) or 'no growth'.

Broth culture outcomes were reported as 'growth from broth culture' or 'no growth'.

All isolates were identified at least to genus level, where possible.

Similar organisms isolated from both bone and swab cultures were not compared for unique identity. Bio-typing, susceptibility testing or molecular typing was not performed.

Data collection

The data was stored in the laboratory information system (LIS), Omni-Lab Version 9.4.2 (supported by Integrated Software Solutions), used for laboratory reporting.

Data for this review was retrieved using a structural query language (SQL) report and deposited in an Excel spreadsheet (Microsoft Office 2003).

Data were retrieved for the 7-year period 1st January 2001 to 31st December 2007.

Results

Between January 2001 and December 2007, 3,439 samples were received from the NSW Bone Bank from 1,331 patient episodes, comprising 1,277 swabs and 2,162 bone fragments. No growth was obtained from 84.1% (1,119/1,331) of episodes with growth obtained in 15.9% (212/1,331) of episodes from at least a swab or bone fragment received.

No growth was reported from 95.1% (3,269/3,439) of samples. Growth was detected from a total of 4.9% (170/3,439) of samples, 8.2% (105/1,277) from swab samples and 3.0% (65/2,162) from bone fragments.

Growth from direct culture of swab on solid media was detected in 0.7% (9/1,277) of samples with only scanty growth reported. Broth cultures of swabs yielded 96 (7.5%) additional isolates. Isolates that grew from direct swab culture also grew the same species from the broth culture.

Table 1 provides a summary of episodes, samples, positive and negative cultures received per year.

Of the 170 positive cultures, there was growth from only one sample of an episode for 90.6% (154/170) of samples and different organisms were isolated from 4.1% (7/170) of cultures from both swab and bone fragments of the same episode. Organisms of the same genus were isolated from both swab and bone samples of the same episode on nine occasions (5.3%).

The greatest numbers of isolates were Gram-positive cocci, predominantly coagulase-negative staphylococci (57.6%, 98/170).

The second group most frequently isolated were Gram-positive bacilli, predominantly diphtheroids (19.4%; 33/170).

Twelve samples had mixed growth, all were coagulase-negative staphylococci plus other Gram-positive species (7.1%; 12/170). Table 2 summarises the organisms isolated from all positive cultures.

Discussion

Bacterial infection is a severe potential complication of bone allograft contamination. To minimise the risk of this complication it is important to ensure all transplanted bone is safe and gamma irradiation of all bone at 25 kGy prior to transplantation is routinely performed at the NSW Bone Bank. Notwithstanding, prophylactic antibiotics are usually given routinely to all patients undergoing orthopaedic implant surgery.

Bioburden assessment determines the estimated numbers of bacteria on an object. A semi-quantitative bioburden assessment is performed on bone surfaces using a swab sampling technique but for bone fragments only qualitative assessment, culture in broth, is possible. Bioburden assessment is not the same as sterility testing. In contrast, sterility testing is performed on batches of products and provides an estimate of the probable sterility of a batch. Sterility tests are destructive tests and also validate the sterilisation process using known quantities of reference organisms from which a D-value has been determined by incremental exposure to the lethal process.

The contamination rate of all samples received in this study of 4.9% appears lower than the rate

Table 1 Summary of number of episodes received, bone swabs and fragments received and culture results from January 2001 to December 2007

Year	Patient episodes	Swabs received	Bone specimens received	Total specimens	Positive cultures (%)	No growth
2001	128	132	278	410	36 (8.8)	374
2002	211	207	419	626	43 (6.9)	583
2003	222	220	449	669	26 (3.9)	643
2004	221	198	401	599	21 (3.5)	578
2005	204	186	274	460	18 (3.9)	442
2006	151	150	152	302	14 (4.6)	288
2007	194	184	189	373	12 (3.2)	361
Total	1,331	1,277	2,162	3,439	170 (4.9)	3,269

Table 2 Positive culture results in bone swabs and fragments from January 2001 to December 2007

Organisms isolated	Swab culture [#]	Bone fragments (Broth only)	Total number
Gram-positive cocci	73	35	108
Anaerobic gram-positive cocci	1		1
Coagulase-negative staphylococci	65 [5]	33	98
Micrococcus sp.	3 [1]	1	4
Staphylococcus aureus[a]	3	1	4
Streptococcus sp.	1		1
Gram-negative bacilli	6	4	10
Acinetobacter sp.		1	1
Brevundimonas vesicularis		1	1
Citrobacter sp.	1		1
Flavobacterium sp.		1	1
Gram-negative bacilli	2	1	3
Gram-negative bacilli, oxidase positive	2		2
Gram-positive bacilli	20	18	38
Diphtheroids	18 [2]	15	33
Bacillus	2	3	5
Other	1	1	2
Cryptococcus laurentil	1		1
Fungus		1	1
Mixed growth	5	7	12
CNS & *Bacillus* sp.	1		1
CNS & Diphtheroids	1	2	3
CNS & Gram-negative bacilli	1		1
CNS & second CNS	2 [1]	5	7
Total (%)	105 [9] (8.2%)	65 (3.0%)	170 (4.9%)
No growth	1172 (91.8%)	2097 (96.8%)	3269 (95.1%)
Total femoral head samples	1277	2162	3439

[a] Three isolates were methicillin sensitive, one was methicillin resistant

# indicates number that grew on direct solid media

Note: 11 samples grew the same organisms in both swab and bone cultures

7 samples grew different organisms in both swab and bone cultures

achieved in other studies, using data from living donors only. Sommerville et al. (2000) revealed a contamination rate of 22% of episodes in a study that tested 4 samples per episode (2 swabs and 2 bone). This rate was reduced to 1.3% if only the surface swab contamination was considered and 12.9% if the surface swab and one bone fragment considered. Vehmeyer et al. (2002) found a contamination rate of 10% using only one swab for each femoral head sample. In the present study, the rate of contamination of the swabs was 8.2% (105/1,277). Journeaux et al. (1999) presented data from femoral heads retrieved from living donors with a contamination rate of 13%, collecting swab and bone samples, Judas et al. (2005) reported 18.2% with no indication of the types of samples collected for testing and Chapman and Villar (1992), 18.5% using broth culture only. Where reported, all studies cultured bone samples directly into a broth medium and swabs onto a combination of broth medium and agar plates.

Qualitative culture of swabs had a contamination rate of 8.2% (105/1,277). The direct culture on agar plates produced growth in 0.7% (9/1,277) of swabs, all with <10 CFU/plate, indicating that only low numbers

of organisms were present after swabbing the femoral head, requiring the more sensitive broth culture to detect their presence. The rate of contamination of the bone fragments was only 3% (65/2,162) but for 90% of episodes where growth was detected only one sample yielded growth. In this study, contaminating organisms were generally species for which virulence is low and there was no gross contamination of femoral heads. It is not possible from the present data set to determine if these organisms were implicated in infection following implantation of the bone. Of the 18 episodes where more than one sample yielded growth, in 11 instances the organisms were of the same genus and different in 7.

As in other studies (Sommerville et al. 2000; Vehmeyer et al. 2002; Journeaux et al. 1999; Judas et al. 2005; Chapman and Villar 1992) the organisms isolated from this study were predominately skin flora. It would be difficult to determine the source of contamination in these samples. Contamination may originate from the patient, at the time of retrieval or from the laboratory during processing. Samples of bone swabs, chips and tissue are collected in the aseptic environments of operating theatres and are immediately sampled and packaged before leaving that space. In the laboratory, samples are processed in a Class II biological safety cabinet.

The femoral head surface swab collected at the time of retrieval represents only a small surface area of the femoral head. Swabbing techniques are not standardised and it would be impossible to swab the entire area of the femoral head. Culture of the whole femoral head is not realistic. The recovery of organisms from swabbing may also be influenced by the type of swab used with some swabs performing better in organism recovery studies than others (Perry and Ballou 1997; Stoner et al. 2008; Perry 1997). Orthopaedic patients generally receive intra-operative prophylactic antibiotics which may also influence sampling results.

Validation studies previously performed in this laboratory (data not shown) detected the growth of low inocula of *Bacteroides fragilis* (12 CFU) and *Staphylococcus epidermidis* (30 CFU) using both solid and enrichment media. While the organisms that are most commonly associated with orthopaedic surgical infections were readily isolated from the media using techniques described, it is possible that some forms of contamination by organisms with specific or fastidious growth requirements may not have been detected. Techniques not requiring the growth of organisms may prove useful in the future. Post-allograft transplantation infections have been documented such as in 2000 where four patients acquired post-surgical septic arthritis associated with bone-tendon allografts which had been irradiated at an indeterminate level (Centres for Disease Control and Prevention 2001) and in 2004 a 23-year old man died of *Clostridium sordellii* sepsis after receiving an un-irradiated allograft (Kainer et al. 2004). These infections emphasised the need for stringent patient donor screening and quality control and improved regulations and processes for musculoskeletal tissue transplant.

All Australian medical microbiology diagnostic laboratories are accredited to AS 4633-2004—Medical Laboratories by the National Association of Testing Authorities (NATA). Assessment by NATA involves an audit every three years headed by a lead assessor and volunteer microbiology peers whose knowledge and understanding of a diagnostic microbiology laboratory is extensive. This laboratory also holds AS/NZS ISO 9001:2000 certification, which examines the quality system of the laboratory and includes many aspects included in the NATA assessment. Attainment of a TGA licence is mandatory to accept samples for bioburden assessment of bone bank samples and is obtained only after an audit by TGA. The TGA audit is led by assessors skilled in the Code of GMP—Human Blood and Tissues (August 2000). This laboratory had an initial audit followed by another 6 months later before changing to an annual audit after 3 years and now to an audit every 2 years. Bone banks must also be audited by TGA and consequently the laboratory is involved indirectly as evidence has to be provided for laboratory related issues that are raised. If the laboratory provides services to several bone banks, the laboratory will be indirectly audited quite frequently. After being accredited, certified and licensed by three bodies, the laboratory benefits from a robust quality system and process.

The bone bank and the laboratory must ensure all processes and methods are validated prior to the commencement of processing of bone bank samples. As the bone banks are located at other sites remote from the laboratory, issues such as swab validation, transport time and transport temperature must be validated. A 5-day incubation period for the bacterial

investigation of clinical tissue samples has been accepted however the incubation period for bone bank samples is yet to be agreed (Murray et al. 2007). It is important that the laboratory is aware of the bone retrieval process as it has been documented that bone banks have used an antibiotic rinse solution before sending bone to the laboratory for testing—this may have a bacteriostatic affect on any organisms present (Kainer et al. 2004; Ireland and Spelman 2005).

James et al. (2004) found that there was no statistically significant correlation in positive culture results and post-transplant infections in the donor and this has been reflected in other studies (Sommerville et al. 2000). There is no common agreement which organisms would lead to automatic exclusion of the use of the bone, although general agreement has been reached on spore forming bacteria such as *Clostridium species* (Ireland and Spelman 2005; Kainer et al. 2004). As false negative culture results can occur, a sterilisation step prior to transplant adds another safeguard to the process. Post sterility assessment is not practical.

At the NSW Bone Bank, bone is gamma irradiated before transplantation. There is however still much discussion on the amount of irradiation required and any detrimental effects to the bone itself (Kainer et al. 2004; Eastlund 2006; Nguyen and Morgan 2007; Balsly et al. 2008). The use of irradiation has also been shown to inactivate many viruses and can be used as part of the quality process of bone banks to safeguard against ineffective screening processes (Pruss et al. 2001). In 1984 a woman received an unsterilised femoral head from a poorly screened donor and became positive for HIV antibody, subsequently developing AIDS (Centres for Disease Control and Prevention 1988). Other studies have suggested a cheaper and faster method of femoral head sterilisation using a domestic microwave as an effective alternate method to gamma irradiation (Dunsmuir and Gallacher 2003) while others have evaluated the use of ethylene oxide on bone grafts (Arizono et al. 1994).

The use of non-culture techniques are being investigated to improve on traditional culture methods in regards to turnaround times and sensitivity. Unusual and new species are being found in bone and joint infections when using a 16S rRNA gene PCR assay followed by sequencing but this assay is being used in patients highly suspected of infection and is complementary to traditional culture (Fenollar et al. 2006). Dempsey et al (2007) found a wide range of organisms within biofilms of clinically infected and non-infected prosthetic hip revisions in both culture and 16s rRNA methods. However, many were found by 16s rRNA alone and it was unknown whether these were viable. Moojen et al (2007) concluded that a combined polymerase chain reaction—reverse line blot hybridization was more sensitive than routine culture in 31 patients undergoing orthopaedic surgery for aseptic and septic indications. Further evaluations need to be performed in this area for bone allografts with issues such as the need for susceptibility testing, reliability of public nucleotide sequencing databases, standardised methods for bioburden assessment, increased costs to laboratories and the clinical relevance of the unusual and environmental organisms being found.

Conclusion

We have reviewed 3,439 bone bank samples over a 7-year period and found a contamination rate of 4.9%. There is no standard method of determining bioburden assessment in allograft samples from living donors and this was reflected in the differing contamination rates found in other evaluations, although the range of organisms isolated were similar. Broth culture is an essential part of organism recovery from swab and bone samples with a greater number of organisms recovered from the broth culture of swabs. The attainment of a TGA licence by a laboratory requires stringent adherence to guidelines, documentation and protocols with regular interaction with bone bank staff. This has ensured all bone banks and associated laboratories have a robust quality system in place which can only improve processes in place. Gamma irradiation of bone prior to transplant provides an additional final safeguard however the dosage still remains under discussion although 25 kGy appears to be accepted in Australia. Further investigation is required in the use and value of non-culture techniques which may reduce the incubation period required and increase sensitivity of testing. Bioburden assessment is a small but integral part of the allograft bone process which together with the bone bank quality processes, the investigation of blood-borne infectious diseases, gamma irradiation and prophylactic antibiotics all combining to provide safe allograft bone for transplant.