Contamination of primary total hip replacements in standard and ultra-clean operating theaters detected by the polymerase chain reaction

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Background **Many organisms that are responsible for low-grade infection after total hip replacement (THR) are not recognized by routine culture.**

Patients and methods **We examined wound contamination during primary total hip replacement performed in standard and ultra-clean operating theaters.**

20 THRs were performed in each type of theater. Paired tissue specimens taken at the beginning and end of surgery were analyzed by bacterial culture and for the presence of bacterial DNA by the polymerase chain reaction (PCR). In total, 160 specimens (80 for culture, 80 for PCR) from 40 THRs were tested.

Results **In standard theaters, none of the 20 specimens taken at the start of surgery were positive by culture, but 3 were positive by PCR (15%). Of the 20 specimens taken at the end of surgery, 2 were positive by enriched culture and 9 were positive by PCR. All specimens positive by culture were also positive by PCR.**

In ultra-clean theaters, none of the 20 specimens taken at the start of surgery were positive by culture, but 2 were positive by PCR. Of the 20 specimens taken at the end of surgery, none were positive by culture, but 6 were positive by PCR. All specimens that were positive by culture were positive by PCR.

Interpretation **Wound contamination of primary THR occurs frequently in both standard and ultra-clean operating theaters and contamination is greater at the end of surgery than at the beginning ($p = 0.04$). In this small series, we found no differences in wound contamination between standard and ultra-clean theaters ($p = 0.1$).**

Infection is an infrequent, but devastating complication that can occur after THR. Most infections are thought to occur as a direct result of wound contamination at the time of surgery (Charnley and Eftekhar 1969, Charnley 1972, Nelson et al. 1980). Since THR became a routine procedure in the 1960s, attention has been paid to the need for strict adherence to aseptic technique in preventing wound contamination and deep infection.

One of the most widely used methods of prophylaxis against infection is the ultra-clean (vertical laminar flow) operating theater which, compared to standard theaters, reduces airborne bacterial load and wound contamination as detected by culture techniques (Charnley 1972, Johnston et al. 1987). However, while there is evidence that the use of such enclosures can reduce the incidence of infection after THR when no routine perioperative antibiotics are used (Lidwell et al. 1982, 1984), the evidence in favor of their use with concurrent parenteral antibiotics is less compelling (Marotte et al. 1987).

It is now known that many organisms that are responsible for low-grade infection after THR are not readily identified in routine culture, and may be detectable only by immunofluorescence or by molecular biological techniques that can amplify small quantities of bacterial DNA (Mariani et al. 1996, Tunney et al. 1998, 1999). This would suggest that many THRs considered to have failed from aseptic causes may have in fact failed due to the presence of unrecognized low-

grade sepsis. Potentially, therefore, measures that reduce wound contamination such as ultra-clean theaters may protect against unrecognized low-grade infection as a cause of late failure in THR, inappropriately considered to have failed from aseptic loosening. In this respect, as culture is too insensitive to allow estimation of the true degree of wound contamination at the time of THR, we assessed the wound contamination rate in the region of the implant zone using the polymerase chain reaction (PCR).

Patients and methods

Having obtained institutional ethical committee approval, we recruited patients undergoing primary THR during a 3-year period (1999–2001). We included healthy patients of any age undergoing primary THR for osteoarthrosis (OA), but excluded those with a history of prior joint surgery or infection.

Surgery and specimen retrieval

Surgery was performed in either standard or ultra-clean vertical laminar flow operating theaters. The standard operating theater was a Triple-S Cyclone Centrifugal Fan (Mathew & Yates, Colchester, UK) providing 20 air changes per hour. The ultra-clean operating theater was a semi-enclosed Integral Exflow 90-5 (Howorth Airtech Ltd., Bolton, UK) providing 530 air changes per hour with an air flow of 80 feet per min. Routine air sampling was performed every 6 months according to HTM 2025 guidelines. The mean CFU count for the standard theater was 50 (36–69) CFU/m^3 and 3 (1–5) CFU/m^3 for the ultra-clean theater.

In all surgery, the skin was prepared with an alcoholic iodine or chlorhexidine solution and covered with impervious drapes (Klinidrape, Mölnlycke Health Care AB, Göteborg, Sweden). The area for the incision was covered with an iodine-impregnated adhesive dressing (Ioban, 3M, Bracknell, UK). Surgical attire consisted of impervious gowns (Klinidrape Op-gown 865602, Mölnlycke Health Care AB, Göteborg, Sweden) and double gloves (Biogel, Regent Medical, Cheshire, UK). Masks used were Tecnol fluidshield (Kimberley-Clark, Roswell, Georgia, USA) and surgical hoods were Barrier Brand (Mölnlycke Health Care AB, Göteborg, Sweden).

Two gowned surgeons and one gowned scrub nurse performed the surgery, in all cases through the posterior approach. After the skin had been incised and initial deep dissection had proceeded, two specimens of pericapsular tissue were taken from the posterior joint capsule. Once these tissue specimens had been taken, antibiotic prophylaxis with intravenous cefuroxime or flucloxacillin was given. At the end of the procedure and prior to closure, further specimens of tissue were taken from the same pericapsular region. Immediately after retrieval, each specimen was separated into two sterile containers. One specimen was taken immediately for Gram stain and culture and the other was frozen at –80°C for PCR at a future date.

Bacterial culture

For aerobic culture, blood agar, blood chocolate agar and 15-lactose electrolyte-deficient (CLED) agar plates (Public Health Laboratories Media Services, Leeds, UK) were used. Samples were incubated in 5% CO_2 at 37°C for 5 days. For anaerobic culture, blood agar plates with and without neomycin were used. Samples were incubated under a deoxygenated atmosphere (5% CO_2, 5% H_2, remainder N_2) at 37°C for 5 days. For enriched culture, a nutrient agar broth with dried minced meat at 37°C was used. Samples were incubated for 5 days and then subcultured onto blood chocolate agar plates for a further 5 days. The detection of one or more bacterial colonies per plate was considered to be a positive culture.

Polymerase chain reaction

We performed all procedures in a purpose-built nucleic acid extraction facility. Total nucleic acid was extracted from 100 μL of the sample using the guanidinium isothiocyanate/silica method (Boom et al. 1990). Nucleic acid was eluted in 30 μL RNase-free water and stored at –20°C until tested by PCR.

A PCR master mix, containing 5 μL 10× PCR buffer II (Invitrogen, Paisley, UK), 2 μL 50 mM MgCl2, 1 μL of each dNTP (10 mM), 0.2 μL Taq polymerase (5 U/μL) and 29.8 μL RNase-free water per sample was prepared and treated for 30 min with UV radiation. 1 μL of each of the prim-

Number of positive culture and PCR cases from each type of operating theater at the start and end of surgery

	Standard operating theater		Ultra-clean operating theater		P-value	
	Culture	PCR	Culture	PCR	Culture vs. culture	PCR vs. PCR
Start of surgery	0/20	3/20	0/20	2/20	1	1
End of surgery	2/20	9/20	0/20	6/20	0.5	0.1
P-value	0.4	0.04	1	0.2		

ers U3 and RU8 (20 pmol/μL) and 10 μL extracted DNA was added to 40 μL of the master mix (Boom et al. 1990, Read et al. 1997). Two controls, one positive (Staphylococcus epidermidis DNA) and one negative (distilled water), were included for every 8 test specimens.

The PCR conditions used were 94°C for 4 min followed by 35 cycles of 94°C for 20 sec, 60°C for 20 sec and 72°C for 20 sec, with a final elongation step of 4 min at 72°C. 10 μL of each PCR product was run in a 3% agarose gel (Nusieve 3:1; Flowgen, Ashby de la Zouch, UK) in TAE buffer and amplicons of 1032 bp were regarded as indicating a positive result.

The sensitivity of the PCR assay was assessed by spiking sterile synovial fluid with 10^3, 10^2, 10 and 1 colony forming units of Staphylococcus epidermidis per 100 μL of sample prior to DNA extraction and amplification. DNA amplification products were detected at all concentrations tested.

The sensitivity of the PCR assay was determined using standard preparations of Staphylococcus epidermidis and the CFUs of these preparations were determined by viable colony counting.

Statistics

We used the Chi-square test for categorical data and Fisher's exact test where data values were less than 5 in any field.

Results

Standard operating theaters (Table)

40 specimens from 20 THRs were analyzed by both PCR and culture (80 specimens in total). Of the 20 specimens taken at the start of surgery, none was positive by culture but 3 were positive by PCR. Of the 20 specimens taken at the end of surgery, 2 were positive by enriched culture only and 9 were positive by PCR. The contamination rate at the end of surgery was greater than the contamination rate at the start of surgery ($p = 0.04$). All 3 specimens that were positive by PCR at the start of surgery were positive at the end of surgery. All specimens positive by culture were also positive by PCR. The bacterium grown on both culture specimens was a coagulase negative Staphylococcus. Antibiotic sensitivity confirmed susceptibility to aminoglycosides (gentamicin) and vancomycin, but resistance to penicillins (flucloxacillin, amoxycillin) and cephalosporins (cefuroxime).

Ultra-clean operating theaters (Table)

40 specimens from 20 THRs were analyzed by both PCR and culture (80 specimens in total). Of the 20 specimens taken at the start of surgery, none was positive by culture but two were positive by PCR. Of the 20 specimens taken at the end of surgery, none was positive by culture but 6 were positive by PCR. All specimens that were positive by PCR at the start of surgery were positive at the end of surgery. The contamination rate at the end of surgery (as detected by PCR) was not statistically different from the contamination rate at the start of surgery ($p = 0.1$); nor was it different from the overall contamination rate seen in standard theaters ($p = 0.3$).

Discussion

It should be emphasized that an appropriate assessment of the true level of bacterial contamination of a wound and its ultimate relevance is not an easy task. We used PCR as a sensitive method of detect-

ing bacterial DNA, as uncultivable organisms may be responsible for a large proportion of joint replacements failing due to unrecognized infection (Mariani et al. 1996, Tunney et al. 1998, 1999). Furthermore, these studies provide evidence that a large proportion of THRs thought to have failed from aseptic causes have actually failed in the presence of unidentified low-grade infection. The relationship between the loosening process and the presence of bacteria at subclinical levels in such cases is, however, unknown and further research is required to evaluate the importance of these findings.

A number of previous studies examining wound contamination have used standard culture techniques by either air sampling or landing 'mode' as a method of assessing potential wound contamination (Lidwell et al. 1983). Neither of these methods, however, directly correlates with wound contamination and they are at best surrogate measures representing the degree of air contamination at the point of sampling, which may be some distance away. The use of gloved hands, swabs and numerous instruments during the surgical procedure is likely to increase the bacterial load delivered to the wound (Davis et al.1999). In this scenario, the direct culture of tissue or taking of bacterial swabs is flawed, in that many organisms may be uncultivable. Tissue penetration of the antibiotics given during surgery may further reduce the possibility of culturing those organisms with some sensitivity to the antibiotic used. The use of molecular techniques for bacterial detection in these situations would thus seem to be an attractive alternative.

While prior studies have shown that ultra-clean theaters can reduce the airborne and wound contamination rate (as detected by culture) as compared to standard theaters, the evidence arguing for their use with concurrent parenteral antibiotic usage is not compelling. Two studies examining this issue, both of which had significant methodological problems (Lidwell et al. 1983, Marotte et al. 1987), failed to show a difference in the overall infection rates between the two types of theater. Bearing in mind that many revision THRs, inappropriately labeled as 'aseptically loose', may in fact have unrecognised low-grade sepsis, a study examining the overall revision rate between the two types of theater would be more appropriate.

One weakness of PCR is that it is used as a surrogate measure for the presence of viable bacteria, which in turn is a surrogate measure of the potential for such bacteria to cause deep infection. It is unfortunate that the technique we used, as a direct result of its extreme sensitivity, resulted in a binary answer (yes or no) to the question of presence of bacterial DNA without providing quantitative results. In this respect, therefore, it may be reasonably argued that using our threshold level of 10 bacteria per cm^3 of tissue or fluid to detect bacterial DNA omits important quantitative information. A lower sensitivity level (×1000) as was used in a previous study (Tunney et al. 1999) may have allowed a quantitative assessment, but would have sacrificed sensitivity in our laminar flow operating theaters where the air count was 3 CFU/m^3. As our aim was to be as sensitive as possible, the former method was chosen.

An additional limitation of our study is that the areas sampled may be reasonably argued to be unlikely to correlate with contamination of the wound as a whole. In this regard, however, we consistently evaluated specimens from the pericapsular region with the assumption that it is these areas, closely associated with the implant, that are most important. The presence or absence of larger numbers of bacteria within the most proximal or distal end of the wound would seem less relevant than a few near the implant zone.

No competing interests declared.

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