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Prevalence and quantification of contamination of knitted cotton outer gloves during hip and knee arthroplasty surgery

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Abstract

Introduction Knitted cotton outer gloves offer protection against surgical glove perforation and provide improved grip on instruments. These gloves absorb blood and other fluids during surgery, and may therefore also accumulate contaminating bacteria. To date, there is no published data on microbial contamination of such gloves during surgery.

Methods Knitted cotton outer gloves used in primary and revision hip and knee arthroplasty from two Swiss hospitals were analysed by quantitative bacteriology. Samples were subjected to sonication and vortexing, followed by membrane filtration of the sonicate. Membranes were incubated under aerobic and anaerobic culture conditions, respectively, for 21 days. Total microbial load for each pair of gloves was determined by colony-forming units (CFU) count. Strain identification was performed with MALDI-TOF.

Results A total of 43 pairs of gloves were collected from continuous series of surgeries. Under aerobic culture conditions, total CFU counts ranged 0–1103, 25 (58%) samples remaining sterile, and 4 (9%) yielding > 100 CFU. Under anaerobic culture conditions, total CFU counts ranged 0–3579, 22 (51%) samples remaining sterile, 6 (14%) yielding > 100 CFU. The only covariate significantly associated with the level of contamination was the provider hospital ($p < 0.0001$ for aerobic and $p = 0.007$ for anaerobic cultures). Strain identification revealed only skin commensals, mainly coagulase-negative staphylococci and *Propionibacterium spp*.

Conclusion While contamination of surgical latex gloves is a well-known issue, no study has examined so far contamination of knitted cotton outer gloves. No or very low microbial contamination could be identified in the majority of the knitted cotton outer gloves assayed. However, a relevant proportion showed contamination far higher than estimated minimal thresholds for implant-associated infection. Clinical relevance of these findings remains to be established.

Keywords Surgical gloves · Knitted gloves · Contamination · Arthroplasty

Introduction

Knitted cotton gloves (Fig. 1) are known to offer protection against perforation of surgical gloves by instruments, sharp bone fragments or when tying sutures [1–3]. They perform better than two or three layers of latex gloves regarding the risk of perforation of the innermost gloves [1–3]. They are appreciated by many orthopaedic and trauma surgeons as they provide an improved grip on the instruments that require forceful manipulation when worn over the surgical gloves.

While provided sterile, the gloves absorb blood and other fluids during surgery, and the fibrous fabric might act as a filter, trapping microorganisms that would not be washed off as easily as from latex gloves. Surface properties of the

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Fig. 1 Picture of knitted cotton gloves worn over doubled latex gloves, as used in both participating hospitals. These gloves commonly are available in green or in white. Green often is preferred, being less shiny when illuminated by the operation room lights. Some surgeons prefer wearing the second pair of latex gloves over the knitted cotton gloves

cotton fibres might even allow bacteria to adhere particularly well [4, 5]. As only a very small amount of bacteria is necessary to cause implant-associated infections [6–10]. Perioperative contamination of these gloves might represent a hazard, particularly in long-lasting operations.

While surgical glove perforation has been studied extensively in various settings, evidence about microbial contamination is comparatively scarce [2, 3, 11–15]. To the best of our knowledge, there is no data available in the literature regarding contamination of knitted cotton outer gloves, and data from studies about contamination of latex gloves cannot be transposed to cotton gloves for various methodological reasons [11, 12, 14]. Specifically, only contact cultures of the fingertips were analysed, under aerobic culture conditions only, and culture time did not exceed 48 h, while microorganisms with low virulence implicated in implant-related infections require longer incubation times to be identifiable [11, 12, 14, 16–19]. This study aims at quantifying contamination over time of knitted outer gloves during hip and knee arthroplasty surgery. To overcome weaknesses of previous studies, whole gloves have been analysed, using both aerobic and anaerobic culture conditions, with prolonged incubation.

Methods

Two Swiss hospitals participated in the study. In both hospitals, knitted cotton outer gloves are used routinely. As no modification of the surgical technique was required for this quality control study, and as no patient identification was required for analysis, no particular approval by the regional ethical committee was necessary (BASEC-Nr. Req-2016-00377).

Transport media and sampling

Polycarbonate containers with threaded lid and 500 ml capacity (Tarsons Products, Kolkata, India) were filled under sterile conditions in a clean room corresponding to a class D of the European GMP guidelines with 350 ml of a custom-made cryopreservative fluid. The fluid was composed of Ringer's solution (Ringer-Bichsel, Bichsel, Interlaken, Switzerland) supplemented with 15% glycerol (Hänseler, Herisau, Switzerland). The containers were then sealed and sterilised in a steam autoclave (MST, Belimed Sauter, Sulgen, Switzerland) at 122 °C for 15 min.

Each participating hospital aimed to collect ten pairs of gloves from a consecutive series of primary and revision hip and knee arthroplasty surgeries. Any operation for suspected or proven infection was excluded. The hospital, surgeon, type of operation, prophylactic antibiotics administered, and the use of bone cement were recorded as potential confounders. The time the knitted cotton outer gloves had been put on, and the duration they were worn, were also recorded. To reduce the risk of contamination and sampling errors, the whole pair of knitted gloves was collected immediately after removal by the surgeon. After sampling, the containers were stored at − 80 °C until assaying.

Surgical techniques and aspects

Operation rooms in both hospitals were equipped with vertical laminar airflow ventilation corresponding to DIN 1946-1:2008-12. The personnel wore polyester-cotton clothing reserved to the theatre area with single use hoods and surgical facemasks. Patient positioning was performed in the anaesthesia ready room before entering the operating room. If necessary, hair was removed in the area of the surgical approach with electric clippers.

Hand and forearm disinfection of the surgical team was performed routinely, applying alcoholic skin disinfectants (Sterillium, Bode Chemical, Hamburg, Germany, or Softa-Man, BBraun Medical, Sempach, Switzerland) for at least 90 s. Surgical scrubbing was performed with an alcoholic povidone iodine solution (Betaseptic, Mundipharma Medical, Basel, Switzerland), applied at least three times and left to dry before draping. Sterile drapes and coats fulfilled the European norm EN 13795 (Products from IVF Hartmann, Neuhausen, Switzerland, used in Hospital A, and from Promedical, Glarus, Switzerland, used in Hospital B). Povidone iodine containing adhesive incision draping (Ioban, 3M, Rüschlikon, Switzerland) of adequate size was applied after draping. Surgical latex gloves (Sempermed,

Semperit Technische Produkte, Vienna, Austria, or Bio-Gel, Mölnlycke Health Care, Schlieren, Switzerland) were worn doubled and the outer pair exchanged after draping.

All patients received cefuroxime (Zinacef, GlaxoSmith Kline, Münchenbuchsee, Switzerland) intravenously as perioperative antibiotic prophylaxis. When bone cement was used, it always was Palacos R + G (Heraeus Medical, Zürich, Switzerland). However, while in Hospital A cement always was manipulated with a supplementary pair of latex gloves worn over the knitted cotton gloves, this was not done in Hospital B. There, the knitted cotton outer gloves were discarded after cementing, and sampled at that moment.

In Hospital A, primary hip arthroplasty (pTHA) was performed through an anterior approach in supine position of the patient on a traction table, implanting pressfit cups (Versafitcup, Medacta, Castel San Pietro, Switzerland) and both cementless or cemented stems (AMIStem or Quadra, Medacta). In Hospital B, pTHA was performed through a lateral approach in supine position of the patient, implanting pressfit cups (Fitmore, Zimmer Biomet, Winterthur, Switzerland) and cemented stems (MS-30, Zimmer Biomet).

During the study period, no hip arthroplasty revision surgery was performed in Hospital B. Thus, only samples from Hospital A were available. Revision surgery was performed either through an anterior approach as mentioned above, or in lateral position through a posterior, respectively, a transfemoral approach, depending on case specificities and extension of the revision.

Knee arthroplasty using knitted cotton outer gloves was performed only in Hospital A. The technique was similar for both primary (pTKA) and revision surgery. The patient was placed in supine position, the thigh fixed in a hydraulic leg holder (Legholder, Lacroix IFM, Clamort, France). A standard medial parapatellar approach was used routinely. Implants for primary and revision arthroplasties were from the GMK family (Medacta) with systematic cement fixation of the tibial component and mainly cementless fixation of the femoral component.

Microbiological workup

Microbiological workup of all samples was performed as a single batch. Frozen samples were thawed overnight at 4 °C. The containers were then subjected to a vortexing–sonication–vortexing sequence, performed in the closed polycarbonate transport containers, following clinical standards [20, 21]. Sonication was performed for 5 min with an ultrasound generator operated at a frequency of 40 kHz with a peak ultrasonic output of 640 W (Bandelin Sonorex Super 10P, Bandelin, Berlin, Germany).

Blood agar (BA) plates (Blood Agar Base, Ref. CM0055, Oxoid, Pratteln, Switzerland) for aerobic cultures, and anaerobic blood agar (ABA) plates (Anaerob Basal Agar, Ref. CM0972, Oxoid) for anaerobic cultures, both supplemented with horse blood (Ref. HB034, TCS Bioscience, Buckingham, United Kingdom), were either inoculated with 1 ml of native sonicate fluid, or used as culture medium for the membrane of EZ-Fit filter system (Ref. EFHAW100B, Merck Millipore, Schaffhausen, Switzerland). The filters have a pore size of 0.45 µm and the aspiration pressure was − 0.6 Bar. As the volume to be filtered was limited by obstruction of the membrane by blood and tissue residues contained in the gloves, two times 20 ml of sonicate fluid were filtered, once for aerobic cultures and once for anaerobic cultures each. 5 ml of each sonicate was also added to 10 ml of thioglycolate broth (Ref. B2551, Fluka Sigma–Aldrich, Buchs, Switzerland) as non-quantitative enrichment for samples with culture negative agars.

Both BA and ABA were incubated at 37 °C either aerobically or anaerobically, the latter in a GasPak EZ System with anaerobic sachets (BD Diagnostics, Allschwil, Switzerland). Growth of microorganisms was checked after 1, 3, 7 and 21 days for aerobic culture plates, while anaerobic plates were checked on days 7 and 21 only, to keep oxygen exposure minimal. Colony counts on the agar plates, respectively, on the filters, were extrapolated to a total number of colony-forming units (CFU) for the whole pair of gloves. All manipulations on the samples and the agar plates were performed under a sterile laminar airflow hood (S@FEMATE 1.8 Vision, Ref. LDGCHD, Euro-Clone, Pero, Italy).

Unused sterile transport media from each participating hospital were processed as above as negative controls ($n = 6$). Additionally, positive control samples ($n = 6$) were prepared by direct skin contact and were processed as positive controls.

Phenotypically different colonies were sampled and stored at − 80 °C until identification. After thawing, subculture of the strains was performed using the same culture media and conditions as mentioned above. Resultant colonies were identified by MALDI-TOF (IVD MALDI Biotyper 2.3, Bruker, Billerica, Massachusetts) according to the instructions of the manufacturer. Distribution of strains was then extrapolated from the number of phenotypically identical colonies identified during the CFU count described above.

Statistical analysis

Revision arthroplasties (RevA) from hips (rTHA) and knees (rTKA) have been grouped. Since there is no reliable data available regarding microbial contamination of knitted cotton outer gloves, no sample size estimation could be performed prior to the study, and so the number of samples required for each category from each hospital was set arbitrarily at 10. Results for aerobic and anaerobic culture conditions are considered separately. The contamination

was analysed as absolute CFU counts, but also binarily for both presence/absence of a detectable contamination, and as contamination exceeding 100 CFUs. The null hypothesis was that there was no correlation between contamination by microorganisms and any one of the parameters collected.

Statistical analysis was performed with SPSS Statistics version 22 (IBM Corp., Armonk, NY, USA). As results are non-normally distributed, data are presented as medians with range. Associations have been analysed with the Kruskal–Wallis or the Mann–Whitney *U* tests, depending on data characteristics. Categorial data have been compared with Fisher's exact test or the Chi-squared test, depending on the number of categories. A p value < 0.05 was considered significant, and analysis always was performed two-sided.

Results

A total of 44 samples was collected during the study period. One sample had to be discarded due to possible contamination during laboratory workup. Hospital A provided samples from 10 pTHA, 10 pTKA, 7 rTHA, and 2 rTKA, all of which were performed by six surgeons. Hospital B provided samples from 14 pTHA, which were performed by three surgeons. Three surgeons contributed < 5 operations each, three surgeons five operations each, two surgeons seven operations each, and the final one surgeon had performed eight operations.

Sonication did not produce any visible cavitation effect on the surface of the fabric. As expected, cultures from native fluid mostly remained sterile, due to lack of sensitivity. Thus, only results from filtered samples have been considered. Subcultures of the thioglycolate broth after 21 days did not yield growth in any culture negative Petri plates. Therefore, the agar plate counts from filtered samples are presented. All of the six negative control samples remained sterile, documenting proper preparation of the transport media and laboratory workup. The cultures from the six positive controls yielded median 20 CFU (range 1–32) for aerobic and 678 CFU (range 360–899) for anaerobic culture conditions, respectively.

Total CFU counts for the whole pair of gloves showed a large range, with a distribution obviously skewed to the left, as more than half of the samples remained free of any identifiable contamination (Fig. 2; Tables 1, 2). After aerobic culture, 25 (58%) samples showed no bacterial growth, whereas this was the case for 22 (51%) of the anaerobically cultured samples. For aerobic cultures, CFU median was 0, ranging 0–1103 (25th percentile at 0, 75th percentile at 22). For anaerobic cultures, CFU median was 0, ranging 0–3579 (25th percentile at 0, 75th percentile at 48). Considering identification of contamination by either of both culture methods, only 17 (40%) of all pairs of gloves remained

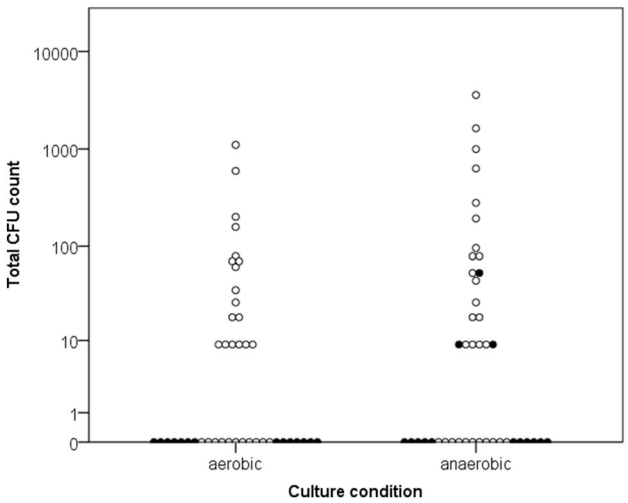

Fig. 2 Distribution of total CFU counts per pair of knitted outer gloves. Results are illustrated separately for aerobic and anaerobic culture methods. Open circles mark results from Hospital A, while dots mark results from Hospital B

sterile. The CFU counts were however low when contamination was identified, as 18 (69% of the 26 culture positive gloves) had CFU counts < 100, whereas only 8 (19% of all) had CFU counts ≥ 100 in either aerobic or anaerobic cultures. In 11 cases (26% of all samples and 42% of the positive samples), only 1 or 2 CFU could be identified by the culture methods, which extrapolated to 9 or 18 CFU for the whole gloves. CFU counts were not significantly different between both culture conditions ($p = 0.41$).

For distribution of parameters such as the hospital involved, type of operation, and use of bone cement, results are summarized in Tables 1 and 2. In all operations, cefuroxime was administered as perioperative antibiotic prophylaxis, and so is not analysed for an outcome effect. A statistically significant difference could be identified between both hospitals for total CFU counts ($p > 0.0001$ for aerobic cultures and $p = 0.007$ for anaerobic cultures) and for the binary analysis of culture positivity ($p > 0.0001$ for aerobic cultures and $p = 0.022$ for anaerobic cultures), but not with the cut-off set at 100 CFU. Subgroup analysis was performed for the above-mentioned parameters within the samples from Hospital A, and to compare samples from pTHA between both hospitals. When considering only pTHA, differences were less marked, but present in the same categories. Considering small numbers and differences between hospitals, analysis of the influence of individual surgeons on contamination is limited. While non-parametric tests identify a statistically significant difference in contamination between the different surgeons, this is to be explained by the differences between both hospitals. Within each hospital, there was no statistically significant difference between the operators. Detailed results are provided in Tables 1 and 2.

Table 1 Quantitative microbiological results from aerobic cultures

	CFU total median (range)	Statistics	contamination negative:positive N:N (%:%)	Statistics	CFU ≤100:>100 N:N (%:%)	Statistics
Total	0 (0–1103)		25:18 (58:42)		39:4 (91:9)	
Hospita l A	9 (0–1103)	$p < 0.0001$	11:18 (38:62)	$p < 0.0001$	25:4 (86:14)	$p = 0.29$
Hospital B	0 (0–0)		14:0 (100:0)		14:0 (100:0)	
Hospital A pTHA only	18 (0–1103)	$p = 0.001$	2:8 (20:80)	$p < 0.0001$	8:2 (80:20)	$p = 0.16$
pTHA	0 (0–1103)		16:8 (67:33)		22:2 (92:8)	
pTKA	0 (0–26)	$p = 0.15$	6:4 (60:40)	$p = 0.22$	10:0 (100:0)	$p = 0.24$
RevA	35 (0–201)		3:6 (33:67)		7:2 (78:22)	
cemented	0 (0–1103)	$p = 0.67$	23:13 (64:36)	$p = 0.11$	34:2 (94:6)	$p = 0.12$
cementless	35 (0–595)		2:5 (29:71)		5:2 (71:29)	
cemented Hospital A	9 (0–1103)	$p = 0.30$	9:13 (41:59)	$p = 0.68$	20:2 (91:9)	$p = 0.24$
cementless Hospital A	35 (0–595)		2:5 (29:71)		5:2 (71:29)	

Table 2 Quantitative microbiological results from anaerobic cultures

	CFU total median (range)	**Statistics**	**contamination negative:positive N:N (%:%)**	**Statistics**	**CFU ≤100:>100 N:N (%:)**	**Statistics**
Total	0 (0–3579)		22:21 (51:49)		37:6 (86:14)	
Hospital A	9 (0–3579)	$p = 0.007$	11:18 (38:62)	$p = 0.022$	23:6 (79:21)	$p = 0.16$
Hospital B	0 (0–53)		11:3 (79:21)		14:0 (100:0)	
Hospital A pTHA only	44 (0–3579)	$p = 0.016$	3:7 (30:70)	$p = 0.035$	7:3 (70:30)	$p = 0.059$
pTHA	0 (0–3579)		14:10 (58:42)		21:3 (88:12)	
pTKA	5 (0–1628)	$p = 0.33$	5:5 (50:50)	$p = 0.44$	9:1 (90:10)	$p = 0.71$
RevA	44 (0–998)		3:6 (33:67)		7:2 (78:22)	
cemented	0 (0–3579)	$p = 0.15$	20:16 (56:44)	$p = 0.24$	32:4 (89:11)	$p = 0.25$
cementless	79 (0–630)		2:5 (29:71)		5:2 (71:29)	
cemented Hospital A	9 (0–3579)	$p = 0.47$	9:13 (41:59)	$p = 0.68$	18:4 (82:18)	$p = 0.61$
cementless Hospital A	79 (0–630)		2:5 (29:71)		5:2 (71:29)	

No correlation could be identified between glove wearing time and the number of CFU (linear regression $r^2 = 0.009$ for both culture techniques). Results are illustrated in Fig. 3. PTHA lasted median 73 min (range 31–184 min), pTKA median 78 min (range 59–95 min), and RevA median 83 min (range 59–108 min). There also was no identifiable correlation between the number of CFU and the time of the day of the operation (linear regression $r^2 = 0.058$ for aerobic, and $r^2 = 0.013$ for anaerobic cultures, respectively).

Bacterial strains identified on the samples are summarized in Table 3. No fungi were cultured. All bacterial strains identified are skin commensals. Strictly anaerobic bacteria such as *Propionibacterium spp* were solely identified under anaerobic culture conditions, while strictly aerobic *Micrococcus spp* and *Bacillus spp* grew only under aerobic culture conditions. Some facultative anaerobic bacteria, either coagulase-negative staphylococci or coryneform bacteria, were also identified only under anaerobic culture conditions. However, these strains could all be subcultured under aerobic conditions, even if some required repeated culturing.

Discussion

Knitted cotton outer gloves (Fig. 1) are popular in orthopaedic and trauma surgery, as they offer the best protection available against perforations of the surgical gloves by

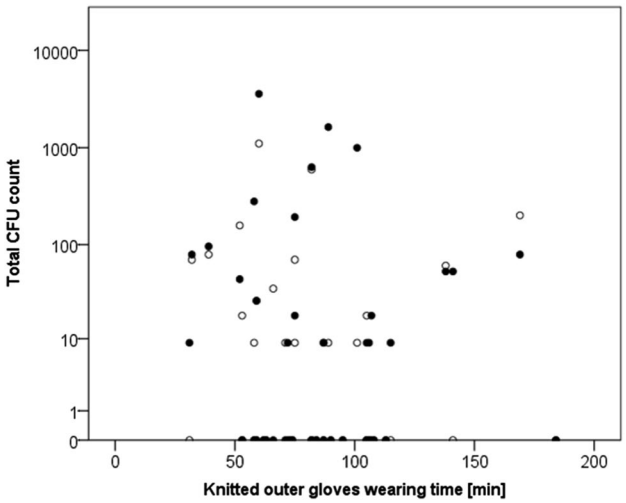

Fig. 3 Distribution of total CFU counts per pair of knitted outer gloves as a function of wearing time. Open circles mark results from aerobic culture methods, while dots mark results from anaerobic culture methods. Note that > 50% of the samples had no identifiable contamination and stack on the abscissa. No statistically significant correlation could be identified between wearing time and the amount of CFU identified, even on subgroup analysis

Table 3 Results of strain identification, as obtained through MALDI-TOF assaying of phenotypically different colonies

Species	Aerobic, *n* (%)	Anaerobic, *n* (%)
S. epidermidis	122 (43.4)	96 (10.0)
S. hominis	8(28.8)	(0.3)
S. warneri	2(9.3)	(0.6)
S. capitis	1 (0.4)	6 (0.6)
S. haemolyticus	8 (2.8)	
S. caprae	1 (0.4)	
Corynebacterium spp	21 (7.5)	30 (3.1)
Corynebacterium tuberculostearicum	13 (4.6)	3 (0.3)
Micrococcus luteus	5 (1.8)	
Kytococcus	2 (0.7)	
Bacillus spp	1 (0.4)	
P. granulosum		409 (42.8)
P. acnes		369 (38.6)
P. avidum		33 (3.5)
Propionibacterium spp		1 (0.1)

Numbers indicate the raw number of colonies identified on the agar plates, respectively, the filters, without extrapolation for the whole volume of fluid/pair of knitted gloves

sharp instruments, bone fragments or when tying sutures [1–3]. There are many manipulations in orthopaedic and trauma surgery associated with a high risk of perforation of latex gloves [12, 15, 22]. The increased grip offered by knitted cotton outer gloves is also appreciated when handling instruments requiring forceful manipulation. To the best of our knowledge, this is the first study examining the contamination of these gloves during primary and revision hip and knee arthroplasty surgery. Slightly more than half of cultures remained sterile (51% for aerobic and 58% for anaerobic cultures), the rest showing identifiable contamination, with CFU counts of up to 1103 for aerobic and up to 3579 for anaerobic cultures. Electron-beam microscopy images illustrating contamination of the knitted cotton gloves are shown in Fig. 4.

Binary transformation of the results was performed as the distribution of CFU counts was skewed (Results in Tables 1, 2). Then, it appears clearly that contamination with potential clinical relevance could be identified in a significant proportion of the samples. A cut-off has been set at 100 CFU, as experimental and clinical data indicate that even as few as some hundreds of bacterial CFU are enough to cause implant-associated infections [6–10]. Values above 100 CFU/pair of gloves could be identified in up to 19% of the samples examined, considering both culture methods together, with individual values above 1,000 CFU, particularly for anaerobic bacteria (Fig. 2; Tables 1, 2). Of course, contamination of the gloves cannot be extrapolated to contamination of the implants, or clinical infection, since the prosthetic components are usually inserted with reduced contact or manipulation with setting instruments and impactors only. Furthermore, clinical follow-up data cannot be provided, particularly as the delay required to detect low-grade infections goes far beyond the scope of this study. Considering the amount of bacteria detected on some of the gloves assayed, it might be of interest to add the parameter use of knitted cotton outer gloves in large studies investigating the infection risk in hip and knee arthroplasty. As wound contamination during arthroplasty in modern operation rooms is low and at the limit of sensitivity even when using filter techniques, examining surrogate parameters might not be sufficient to provide adequate answers.

The results observed indicate sound study methods. The negative controls remained sterile. Results on the other end of the spectrum of numbers of CFU remained within the detectable range, be it for the samples with the highest contamination, or the positive controls. Also samples have been gathered in two hospitals to reduce the bias from local specificities, even if in the end only the difference between both hospitals appeared as being significant. Data from available studies of contamination of latex gloves cannot be transposed to knitted cotton outer gloves for various methodological reasons. In these studies, sampling had been performed only on fingertips, only contact samples have been analysed, and incubation time was too short to identify slow-growing microorganisms [11–14, 17, 18]. Many efforts have been made to optimise the sensitivity in this study: the whole pair of gloves had been worked up to reduce sampling errors and

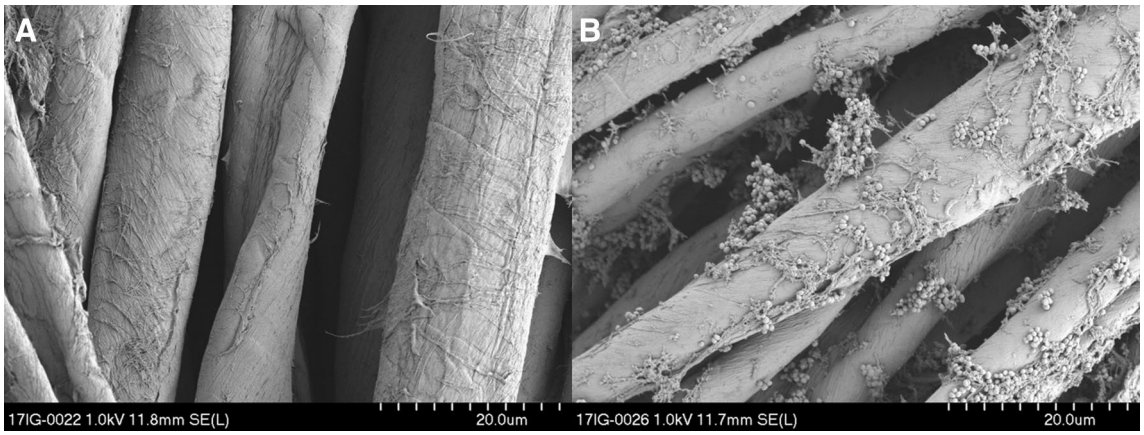

Fig. 4 High magnification scanning electron microscopy images of a negative (**a**) and an artificially contaminated positive (**b**) control samples. Contamination was performed by contact with the skin on a forearm. The number of CFU identified on positive controls corresponded to the upper range of the clinical samples. Note the presence of multiple cocci in (**b**), partially surrounded by protein agglomerates, respectively, beginning biofilm

risk of contamination due to manipulations; vortexing and sonication have been applied to dislodge residues from the fabric [20, 21] a filtration system had been used to increase sensitivity; incubation was performed over 21 days for both aerobic and anaerobic culture media, with further subculture of a thioglycolate broth in case the agar plates remained sterile [17–19] and finally, strain identification was performed by MALDI-TOF. Comparison of contamination of the knitted cotton outer gloves with contamination of latex gloves would not have been feasible in the setting of this study. As knitted cotton is not waterproof or impervious to bacteria, contamination of the underlying latex gloves may be expected to be qualitatively similar. A control group from identical operations without knitted cotton outer gloves would have been unethical before results from this study were available, as the use of knitted cotton outer glove was routine and not using them would have exposed patients and staff to a known risk of contamination through glove perforation [1–3, 15]. Last but not least, microbiological workup of latex gloves poses major additional challenges, as only the outer surface of the gloves should be cultured. Methodological differences limit comparability anyway.

Despite these efforts, all bacteria present on the knitted gloves might not have been identified. As mentioned, sonication did not produce any cavitation bubbles on the surface of the fabric, probably due to the softness of the cotton. However, duration and intensity of sonication recommended in clinical practice [20, 21, 23] have not been modified for the following reasons. Biofilm should not have time to form between sampling and workup [4]. Bacterial clumping might however have occurred, as it happens in vitro within hours in synovial fluid [24], considering operation and thawing time. This might have reduced the number of CFU identified. Increasing the duration of sonication would have introduced an arbitrary risk of devitalisation of the bacteria [23]. The fluid might not have been homogenised perfectly as the gloves had not been removed to minimize manipulations and risk of contamination. This might introduce a sampling error of unknown importance, to which adds another potential sampling error as only part of the fluid could be filtered, due to relatively quick obstruction of the filter system. As some facultative anaerobes were identified initially only in anaerobic cultures, there definitely was a sampling error. Considering the very low contamination of most samples and the technical issues with the filtering system, such variability is of no surprise but should not influence the main conclusions of the study. PCR quantification of bacteria was not performed as the gloves might be contaminated by dead microorganisms, residual disinfectant on the patient's skin and perioperative rinsing of the wound being potential sources of false positive results.

As expected, the microorganisms identified are all skin commensals (Table 3) [11–14, 25]. Highly virulent bacteria such as *S. aureus* or *P. aeruginosa* were not identified in this series, as other authors reported from studies of latex gloves [12, 25]. Strictly anaerobic bacteria such as *Propionibacterium spp* were identified only under anaerobic culture conditions, while strictly aerobic bacteria such as *Micrococcus spp* and *Bacillus spp* were solely identified in aerobic cultures, as expected. This is an indication of proper microbiological culture methods. Some facultative anaerobic bacteria, mainly coagulase-negative staphylococci, but also coryneform bacteria, were however identified initially only under anaerobic conditions. All these strains could finally be regrown under aerobic conditions, even if some required repeated culturing. No atypical anaerobes were present in this series [26]. Of note, most *Propionibacterium* strains identified were not *P. acnes*, whereas the other species

identified here are extremely rare in periprosthetic joint infections [27].

The only cofactor examined in this study which had a statistically significant association with contamination, was the participating hospital. Contamination was lower in samples from Hospital B than in those from Hospital A. This was true for total CFU counts ($p > 0.0001$ for aerobic cultures and $p = 0.007$ for anaerobic cultures), and for the binary analysis of culture positivity ($p > 0.0001$ for aerobic cultures and $p = 0.022$ for anaerobic cultures), but not with the cut-off set at 100 CFU. Small numbers limit further analysis. Only suppositions can be made regarding the cause. One explanation might be that in Hospital B, where no or only minimal contamination was detected, the bone cement got in contact with the knitted outer gloves, which were then discarded after cementation. The bone cement used always contained gentamicin. As gentamicin mainly requires the presence of oxygen to be effective [28] this might be an explanation why microorganisms were detected only under anaerobic culture conditions, if any were detected at all in samples from Hospital B (Fig. 1; Tables 1, 2). In Hospital A, a supplementary pair of latex gloves was worn over the knitted gloves when handling cement, thus no cement or antibiotic exposure of the cotton gloves occurred. This may explain the low culture positive rate at Hospital B, even if the exposure to antibiotics would be expected to be short and quickly diluted in the transport fluid. A second possible explanation might be the operation room ventilation. Increasing amounts of data point out laminar airflow as a risk factor for surgical site infection after arthroplasty [29, 30] and therefore, positioning of the patient and surgical approach might be suspected as possible explanation for the observed difference in contamination between both hospitals. While for all operations performed in Hospital A the laminar airflow blew vertically into the wound, a lateral approach to the hip in supine position was used Hospital B, avoiding this exposure.

As bacterial growth is time-dependant, it was expected that microbial contamination of the knitted outer gloves would increase with wearing time. This hypothesis could however not be confirmed in our study (Fig. 2). On the contrary, the risk of perforation of latex gloves, and thus contamination, increases with wearing time [1, 12, 14, 22]. Nevertheless, duration of surgery is not identified consistently as a risk factor for surgical site infection after arthroplasty [30, 31]. A follow-on study will examine the effect of wetting the knitted cotton gloves with disinfectant, instead of crystalloid solution. An antibacterial coating might possibly reduce the contamination, as already demonstrated effectively for latex gloves, but represents a solution more complicated to implement [32].

In conclusion, while the use of knitted cotton outer gloves does have proven advantages in orthopaedic and trauma surgery, offering the best protection available against perforation of latex gloves, it also carries a relevant risk of significant intraoperative contamination by skin commensals in hip and knee arthroplasty surgery. Surprisingly, wearing time was not a determining factor. Iterative glove exchange thus cannot be recommended as prevention. While most gloves assayed remained sterile or showed very low bacterial contamination, some CFU counts were far higher than threshold values known to be sufficient to cause implant-associated infections. The clinical relevance of these findings remains to be determined. Nevertheless, it might be recommended to avoid manipulation of implants with these gloves, but this is a general rule always valid even with latex gloves. The use of knitted cotton outer gloves is a parameter that should be considered in larger studies about infection rates in hip and knee arthroplasty. Comparison with contamination of latex gloves, which are known to be contaminated during surgery, is limited or impossible, due to major methodological differences between this study and other publications. However, as knitted cotton is not waterproof or impervious to bacteria, contamination of the underlying latex gloves must be expected whenever the knitted cotton outer gloves appear contaminated, even if differing physicochemical properties between materials might result in some quantitative differences.

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