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Hair removal with a clipper and microbial colonisation prior to knee arthroplasty: a randomised controlled trial

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SUMMARY

Background: Despite the widely reported success of knee arthroplasty, studies show that 1.6—3 % of patients undergo revision within the first postoperative year predominantly due to infection. Preoperative skin preparation may potentially decrease the bacterial load and consequently, the risk of periprosthetic joint infections. The effects of hair removal on prosthetic joint infection are inconsistent. Our primary aim was to investigate if hair removal with a clipper influenced skin colonisation and bacterial composition.

Methods: Forty Caucasian male participants who were planned to undergo knee arthroplasty, (mean age 63.8 years), were included. Patients were randomised to hair removal in a within-person study design. As a control, the opposite leg of the patient was used. Swabs were collected prior to hair removal (baseline), immediately after hair removal (Day 0), and with follow-up after one and seven days.

Results: The intervention showed significant decrease in mean log colony-forming units per. cm^2 from baseline 2.97 to 2.67 ($P<0.01$) immediately after hair removal and sustained at Day 1 ($P=0.01$). At Day 7, the mean was non-significant compared to baseline. The control group did not show any decrease of skin microbiota at follow-up on Day 0, 1 or 7. No significant differences within the bacterial composition were found between the intervention and control leg at baseline among the six most prevalent detected bacterial species: *Staphylococcus epidermidis, Micrococcus luteus, S. hominis, S. capitis,*

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S. haemolyticus and S. aureus. The study did not find any changes in the bacterial composition over time.

Conclusion: Hair removal with a clipper within 24 hours prior to surgery causes a significant non-selective reduction in skin colonisation.

Introduction

Despite the reported success of knee arthroplasty (KA), Danish and European studies show that a range from 1.6-3% of patients undergo further surgical revision within the first postoperative year [1—5]. The usual indication for early revision is periprosthetic joint infection (PJI) [1—3,6]. PJI is considered a devastating and complex complication associated with risk of severe morbidities, possible loss of independence, prolonged hospitalisation, and increased healthcare costs [7—9].

Causes for PJI are multifaceted and affected by both intrinsic and extrinsic factors. Studies show that age, male sex, obesity, smoking, and increased alcohol consumption as well as the comorbidities diabetes mellitus and rheumatoid arthritis, increase the risk of infection [3,10]. In addition, perioperative factors such as skin preparation, duration of surgery, bleeding complications and microbial contamination are known to affect the risk [3,10,11].

Staphylococcus aureus and *Staphylococcus epidermidis* originating from the patient's commensal skin flora have been identified as the most common causative organisms related in PJI [7,10]. Therefore, skin preparation before surgery to decrease the bacterial load is considered relevant to reduce the risk of PJI. Several preoperative interventions such as body washing, skin decontamination with chlorhexidine gluconate and the use of antimicrobial incision drape reduce the patient's skin microbiota in the surgical field [12—14]. The WHO recommendations, based on an inconclusive Cochrane review from 2021, recommend that hair removal is not advised as part of skin preparation, but if necessary, it should be performed with a clipper [14,15].

The evidence as to whether removal of hair can reduce the risk of infection remains unresolved. It is an interesting question for several reasons. Due to its growth from follicles beneath the surface of the epidermis, hair represents a tissue component colonised with microorganisms, as is the skin surface [16]. Growth of body hair is known to be of intrapersonal as well as interpersonal variation [17], but in males, testosterone further contributes to an increased growth of terminal hair in the face as well as the chest, back, legs and genitals [18]. This difference in hair growth may explain why men have a significantly higher bacterial contamination in the surgical field and correspondingly an increased risk of infections [3,4,12,19].

Previous studies of hair removal methods using razors, depilatory cream or clipping on PJI have found that hair removal performed with a clipper was the gentlest method for skin preparation [15,17,20,22]. Studies investigating the effect of hair removal with a clipper on PJI, are characterised by low quality due to short follow-up times, time of hair removal, lack of specification of method, location, and control group [13,15,21,22]. The present study applied a randomised controlled design to investigate the effect of hair removal with a clipper on skin colonisation and to investigate if hair removal could lead to changes in the skin microbiota in male patients prior to knee arthroplasty.

Methods

Study design and setting

A non-blinded within-person randomised controlled study design was applied, where the patient acted as his own control for skin swabs taken from both intervention and control sites.

The primary outcome was the between-group difference in colony forming units (CFU) reduction at Day 0, 1 and 7. As a secondary outcome, changes in the skin microbiota composition after hair removal with a clipper were investigated.

The sample collection, bacterial cultures and CFU count were performed by one investigator, while the bacterial identification was performed by another investigator to ensure uniform collection and processing of samples.

Power calculation

Sample size was calculated based on data from Jung *et al.*, 2016 [17], who found a reduction in mean Log10(CFU) from 2.6 $\pm$ 1.27 (SD) to 1.76 $\pm$ 0.8 (SD) from Day 0 to Day 1, due to hair removal with clipping. With a 1.0 SD with log10, 2-tailed $\alpha = 0.05$, a power of 80 % and an effect of 0.84 the sample size should be a total of forty participants due to the 'within person' design.

Ethics statement

The study was considered by the Danish Science Ethics Committee to be a pre-clinical study and waived an approval (J.no. 19084310). The study was conducted in accordance with the Helsinki Declaration [23]. The Orthopedic Surgery Department approved the project and followed GDPR regulations [24]. All participants received oral and written information and provided informed consent. The study did not have any influence on planned surgery, and participants could withdraw at any time.

Bacterial isolates were stored at Costerton Biofilm Center at University of Copenhagen Denmark.

Participants

Participants were from The Orthopedic Surgery Department at Herlev-Gentofte Hospital, Denmark. All measures and swabs were performed prior to planned surgery. Male patients $\geq$ 50 years were eligible for enrollment.

Patients who used systemic or local antibiotics, steroids, moisturizer, had performed hair removal within the previous 30 days, had active skin disease or inflammation, scars in the sample area, were excluded. The participants were required to have a shower no more than 6 hours prior to samples, to simulate local instructions for skin cleaning prior to KA.

Ninety-three patients were screened (Figure 1). Forty Caucasian males, 50—85 years of age volunteered to participate in the period June—October 2020.

Procedure

The hair removal site was determined by randomisation of one of four possible locations: right leg proximal (R1), right leg distal (R2), left leg proximal (L1) and left leg distal (L2) of the patellae (Figure 2). The sample location for hair removal was selected by randomisation, for example R1 with the opposite L1 automatically becoming the control. Baseline samples were then taken from both remaining sites, respectively R2, L2.

Sample sites for intervention and control were maintained throughout all sampling: Day 0, 1 and 7.

Hair removal was performed in a 5x5 cm square centered on the randomised sample site. Hair removal was performed with BD Surgical Clipper 5513E Rechargeable Unit ® (Vernon Hills, IL, USA) calibrated to a hair length of approximately 0.23 mm. After clipping, hair residues were removed with a clean, dry towel. The control leg was equally wiped.

Participants were positioned supine in a hospital bed during sampling. Swabs were collected in midline proximal to patella for the purpose of obtaining changes in skin colonisation as close to the incision site for KA as possible.

Skin swabs were performed prior to hair removal (baseline), immediately after (Day 0), one day after (Day 1) and seven days (Day 7) after hair removal. Swabs were collected from both intervention and control sites at each time point.

Sampling was performed by the 'cylinder-sampling' method [17,25,26] using a sterile stainless-steel cylinder made of AISI 304 certified steel with an inner diameter of 3.8 cm (total sample site of 11.3 cm^2).

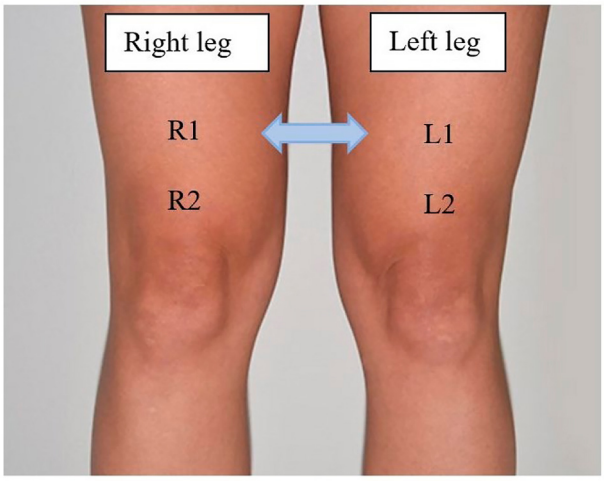

Figure 2. Randomisation sites intervention was performed in randomization between R1, R2, L1, L2. Control was performed in parallel site on opposite legs. Baseline was performed in remaining sites.

The sterile cylinder was placed on the sample site and added with 3.5 ml. sterile water. The area was scrubbed with a sterile swab in even grid formation for one minute. The material was transferred into a sterile tube using a sterile syringe (Figure 3). The procedure was repeated, and material was added to the same tube. Samples were immediately stored on ice for 3—4 hours until further processing.

Culture of skin microbiota and bacterial identification

In ten-fold serial dilutions, 1.0 ml of 7.0 ml suspension was used for CFU count. Bacterial dilutions were plated on 5 % blood agar plates and incubated at 35° C for 48 hours. The CFU were determined by manual cell count [27] and explicated as CFU/cm^2. The bacterial isolates were collected based on

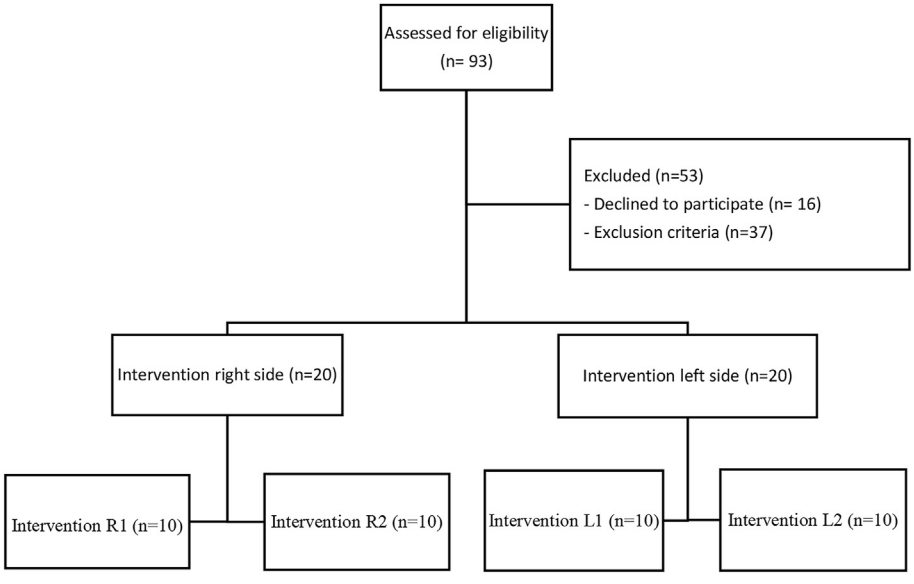

Figure 1. CONSORT flow diagram.

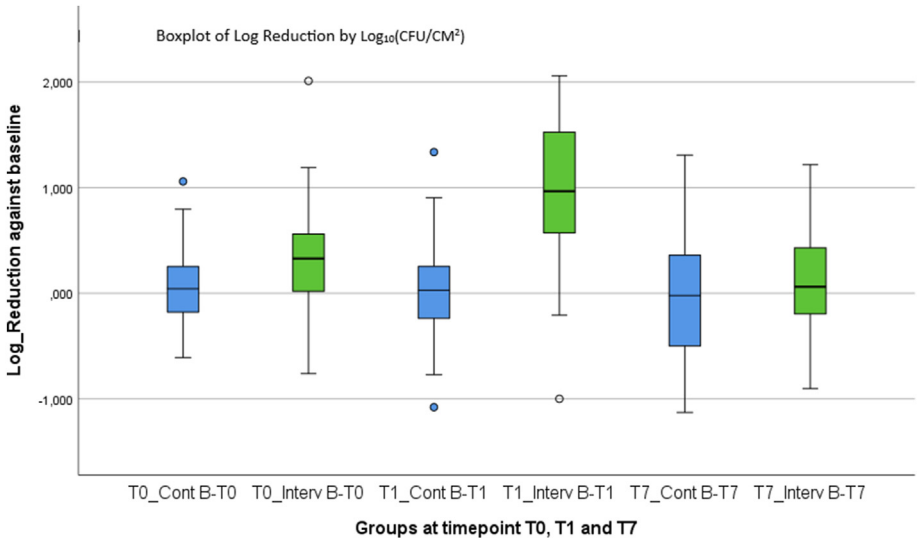

Figure 3. Boxplot of log reduction by $Log_{10}(CFU/CM^2)$.

morphology (form, shape, structure) with one sample per colony and stored at -80° C in LB (Luria-Bertani) broth with 20% glycerol.

A total of 1555 isolates were prepared for matrix-assisted laser desorption-ionization — time of flight mass spectrometry (MALDI-TOF MS®) identification according to the manufacturer's manual (Bruker, Billerica, MA, US). Isolates were identified using a Microflex LF mass spectrometer, Bruker ® Biotyper 3.1 software and BDAL standard library (Bruker) at the department of Clinical Microbiology, Rigshospitalet, Copenhagen. The following analysis includes only isolates achieving a quality score of at least 2.0. Isolates not immediately identified were re-analysed up to four times [28,29]. Furthermore, inclusion of species was limited to those present in a minimum of three patients in the groups of intervention or controls.

Statistics

Primary outcome

Log_{10} reduction (LR_{CFU}) was tested for symmetry with P—P plots to make a parametric comparison of log10 transformed CFU/cm^2 between groups. $LR_{CFU} = log_{10}(CFU/cm^2)_{DayX} - log_{10}(CFU/cm^2)_{baseline}$ were analysed with a paired t-test at Day 0, 1 and 7. Boxplot was applied to visualise the normal distributed parametric log Reduction in control and intervention group at each timepoint.

Microbiological data was classified as paired categorical variables. McNemar's test was used to calculate a possible statistically significant association between as within the two groups at the different time points.

Statistical significance was set at $P \leq 0.05$. Statistical analyses were performed with R, version 4.0.3 [30] and IBM SPSS Statistics for Windows ®, version 28 (IBM Corp., Armonk, N.Y., USA). For randomisation, Procordo Software (Procordo, Copenhagen, Denmark) was used.

Results

All 40 participants attended at every time point (baseline, Day 0, 1 and 7), giving a 100% participation rate. All samples (160 + 160) were collected and handled per protocol. In eight samples, two from the control group and six from the intervention group, no bacteria were detected. Table I provides the participants' characteristics.

Primary outcome

The log transformation of CFU did not reject a normal distribution and allowed us to perform parametric t-tests. The mean count of log transformed CFU (log_{10} CFU/cm^2) changed significantly in the intervention but not the control leg that remained stable over time (Table II). The between groups parametric measures of CFU reduction (LR_{CFU}) revealed significant differences at Day 0 and Day 1 favouring the intervention leg, while no difference was seen between legs at Day 7 (Table II). Figure 3 provides a box plot of parametric LR_{CFU} at Day 0, 1 and Day 7. (A positive value represents a higher reduction of CFU).

Change in microbiota

Overall 1555 isolates were analysed. A quality score below 1.70, resulting in no identification was seen in 32 isolates (2%)

Table I
Patient characteristics.

Participant characteristics, self- reported, *N*=40	Mean (range)
Age, y	66 (50—82)
Weight, kg	92 (68—113)
Height, m	1.8 (1.7—1.9)
Body Mass Index (BMI)[a]	27 (23—31)
Alcohol, week	6 (0—28)
Smoking, day	0 (0—15)
Diabetes mellitus	
IDDM[a]	1
NIDDM[b]	1
No diabetes	38

[a] IDDM Insulin-dependent diabetes mellitus.
[b] NIDDM non-insulin dependent diabetes mellitus.

Table II
CFU_{Mean} and log reduction

Data are presented as log_{10} CFU/cm^2							
Knee allocation	Baseline Mean (SD)	Day 0 Mean (SD)	*P*- value[a]	Day 1 Mean (SD)	*P*- value[a]	Day 7 Mean (SD)	*P*-value[a]
Control	2.90 (0.58)	2.84 (0.60)	0.30	2.88 (0.61)	0.68	2.94 (0.57)	0.72
Intervention	2.97 (0.71)	2.67 (0.76)	<0.01	2.71 (0.63)	0.01	2.83 (0.61)	0.12
LR_{CFU}[b]		0.24 (0.60)		0.94 (0.83)		0.17 (0.59)	
		P=0.02		*P*< 0.01		*P*=0.08	

[a] *Within* group variance from baseline.
[b] *Between* group variance (LR_Intervent. - LR_Control) on log_{10}Reduction of T*x* - Baseline.

and 91 isolates (6%) had a score between 1.71 and 1.99, and it was only possible to identify at genus level.

Overall, 1432 isolates (92,1%) were successfully identified. Following analysis, 362 duplicates of bacterial species within the same sample were excluded from the dataset. The final statistical analysis included 1070 microbiological identifications.

After identifying baseline isolates, a predominance of six different bacterial species emerged in the intervention group (I) and the control group (C), respectively: *Staphylococcus epidermidis* (I:55%; C:70%) and *Micrococcus luteus* (I:63%; C:55%), *Staphylococcus hominis* (I:35%; C: 40%), *Staphylococcus capitis* (I:40%; C:30%), *Staphylococcus haemolyticus* (I:25%; C:42.5%) and *Staphylococcus aureus* (I:15%; C:12.5%). In addition, in a subset of 2.5%—10% of the patients *Staphylococcus caprae, Staphylococcus warneri, Staphylococcus lugdunensis, Corynebacterium amycolatum, Corynebacterium aurimucosum, Acinetobacter lwoffii* and *Bacillus cereus* also occurred. The McNemar's test showed no statistically significant difference between intervention group and control group at baseline in neither the six most often detected species nor the rare ones.

At Day 0 and 1, the same six bacterial species dominated in both the intervention group and the control group, thus, no significant difference was seen between groups. At Day 7, when the regrowth of hair appeared, the commensal community were restored and did not differ significantly between groups (Table III).

Changes over time in the two groups were examined within the most frequently occurring bacterial species (*S. epidermidis, M. luteus, S. hominis, S. capitis, S. haemolyticus and S. aureus*). There was no significant difference in the bacterial composition over time when the baseline species were compared to the findings of Day 0, 1 and 7.

Discussion

This study aimed to investigate the effect of hair removal on changes and variation in the skin microbiota prior to surgery with KA. The significant reduction in CFU immediately after hair removal and following day highlights this potential method in reducing skin commensals prior to surgery. The similar microbial composition in the skin of intervention and control legs suggest that hair removal does not cause selection of specific bacteria such as *S. aureus* and *S. epidermidis*. The low numbers of Corynebacterium species are consistent with findings in a previous study when we observed that the knee was colonised with relatively few culturable bacteria compared with other body sites. [12].

The main benefit was the significant reduction in the number of bacteria. This point was strengthened by the changes in CFU over time, although the reduction was insignificant within a week after hair removal. Our findings are consistent with the findings of Jung *et al.*, 2016 [17], although, their results may have been influenced by a limited number of participants and the lack of a control group, making it difficult to compare directly with our findings.

There is evidence suggesting that hair removal with a razor may be harmful, causing microlesions which form a reservoir for bacteria, and increase the risk of infection. When using a razor, the sharp blade is drawn directly across the skin, whereas the clipper cuts the hair close to but not at the base why the skin is left intact [17]. It should be noted that hair removal is recommended to be performed outside the operating theatre to prevent bringing contaminated hair residue into a sterile environment [31]. The time taken for clipping and the subsequent clean-up has been examined in a previous study, which found that clipping including the clean-up took approximately 4.2 minutes for a lower leg [27]. Hair removal should be considered a relatively small cost compared with the costs associated with PJI.

The individual's skin microbiota is influenced by several different factors (age, sex, health status, skin habitat and anatomical location) [32—33]. Additionally, environmental factors such as occupation, clothing [34], and the individual's use of hygiene products may also influence the microbial composition [33,34].

In our study, some bacterial species were only identified in a few individuals. These findings reflect the individual factors affecting the skin microbiota. However, the significance of the interplay between the host and the skin microbiota is not fully understood. The community composition and the inter-microbial interaction may play a role in the risk of developing surgical wound infections [35].

There were strengths and limitations of this study. Even though, there is a predominance of women among patients who undergo K.A., older men are at a higher risk of complications. Hence in this study, only men over the age of 50 were investigated [19]. Elderly men often have abundant hair growth, which is a potential reservoir for bacteria [16]. Due to sweat production and hormones both sex and age plays a role in the skin microbial composition [16,18]. Ethnicity, social behavior and economic status may also influence the detection of less common species in the skin microbiota [34]. However, this randomised controlled within-person design limited these

Table III
Microbiota

n=40	Intervention n (%)				Control n (%)				*P*-value (McNemar)			
	Baseline n	Day 0 n (%)	Day 1 n (%)	Day 7 n (%)	Baseline n (%)	Day 0 n (%)	Day 1 n (%)	Day 7 n (%)	Baseline n (%)	Day 0 n (%)	Day 1 n (%)	Day 7 n (%)
S. epidermidis	22 (55%)	22 (55%)	28 (70%)	24 (60%)	28 (70%)	29 (72.5%)	24 (60%)	24 (60%)	0.21	0.143	0.344	1
M. luteus	25 (63%)	25 (63%)	12 (30%)	12 (30%)	22 (55%)	22 (55%)	14 (35%)	15 (37.5%)	0.549	0.375	0.727	0.607
S. hominis	14 (35%)	18 (45%)	18 (45%)	12 (30%)	16 (40%)	19 (47.5%)	15 (37.5%)	13 (32.5%)	0.791	1	0.607	1
S. haemolyticus	10 (25%)	15 (38%)	26 (65%)	24 (60%)	17 (42.5)	11 (27.5%)	26 (65%)	26 (65%)	0.065	1	1	0.754
S. capitis	16 (40%)	17 (43%)	13 (33%)	16 (40%)	12 (30%)	14 (35%)	12 (30%)	19 (47.5%)	0.344	0.508	1	1
S. aureus	6 (15%)	5 (13%)	6 (15%)	6 (15%)	5 (12.5%)	8 (20%)	8 (20%)	5 (12.5%)	1	0.375	0.727	1
S. caprae	4 (10%)	2 (5%)	2 (5%)	1 (2.5%)	3 (7.5%)	1 (2.5%)	1 (2.5%)	4 (10%)	1	1	0.625	0.375
S. warneri	2 (5%)	2 (5%)	5 (13%)	4 (10%)	1 (2.5%)	1 (2.5%)	5 (12.5%)	2 (5%)	1	1	1	0.5
S. lugdunensis	3 (7.5%)	2 (5%)	2 (5%)	3 (7.5%)	2 (5%)	5 (12.5%)	4 (10%)	3 (7.5%)	1	0.453	0.625	1
C. amycolatum	4 (10%)	2 (5%)	1 (2.5%)	6 (15%)	2 (5%)	2 (5%)	1 (2.5%)	10 (25%)	0.625	1	1	1
C. aurimucosum	1 (2.5%)	2 (2%)	5 (13%)	2 (5%)	3 (7.5%)	2 (5%)	2 (5%)	1 (2.5%)	0.625	1	0.375	1
A. lwoffii	2 (5%)	3 (7.5)	2 (5%)	5 (13%)	2 (5%)	2 (5%)	2 (5%)	4 (10%)	1	1	1	1
B. cereus	2 (5%)	4 (10%)	1 (2.5%)	5 (13%)	3 (7.5%)	2 (5%)	0 (0%)	6 (15%)	1	0.687	1	1

confounding factors. The study exclusively included Caucasian males. Furthermore, patients with skin disorders, or patients who needed treatment with hormones were excluded. These patients have a certain degree of damaged skin, which may ease penetration of bacteria through the skin barrier [36]. In particular, an increased occurrence of yeasts and *S. aureus* have been observed in the skin microbiota of these patients [36]. Consequently, patients with skin disorders of various kinds may be at increased risk of acquiring a surgical site infection when undergoing K.A. Thus, the choice of patient population in the present study strengthens the internal validity, while decreasing generalisability.

Another noteworthy strength was that sample collection and processing was conducted by two researchers and thereby uniformly handled in every step. The cylinder method used for sampling, performs wet sampling, which releases up to 10 times more bacteria compared with dry sampling, [37]. Additionally, the double scrubbing of the sample site collected up to 97% of the skin microbiota present [38]. Furthermore, a larger sample site, compared with previous studies [17,26] adds strength to the sample collection.

The use of antiseptic disinfectant for skin preparation in the operating theatre is a well-integrated procedure prior to surgery to reduce the risk of PJI [13,32]. What effect the combination of hair removal has in combination with chlorhexidine gluconate and use of antimicrobial incision drape on the contamination in the surgical field needs further investigation. PJI often presents as a late outcome following the intervention, and it is therefore difficult to judge an immediate effect [1,39,40]. Nevertheless, acknowledgement that hair removal can contribute to reduced colonisation and thus potentially a reduced risk of infection is of clinical importance.

Conclusions

Hair removal with a clipper has a transient effect on reducing the microbial colonisation of the skin prior to K.A. in males. The occurrence, variation and potential selection in the skin microbiota did not change significantly over time following hair removal.

The study findings may be relevant to other surgical specialties such as gastrointestinal surgery and thoracic surgery, where hair growth is highly represented on the skin and the prevention of surgical site infection is crucial to achieve a positive clinical outcome.

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Conflict of interest

The authors declare no conflict of interest.

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Credit author statement

Definition, Conceptualization and Ideas; formulation or evolution of overarching research goals and aims (AO, TB, ABH, THH, TM, CF).

Methodology: Development or design of methodology; creation of models (AO, TB, ABH, THH, TM, CF).

Software. Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components (AO, TM, BF, TH, CF).

Validation: Verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs (AO, TB, TM, TH, CF).

Formal analysis: Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data (AO, TM, BF, THH, CF).

Investigation. Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection (THH, CF, AKN, LB).

Resources. Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools (CF, TB, LB, AKN).

Data Curation. Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later reuse (THH, CF, TM, AO, BF).

Writing - Original Draft. Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation) (THH, CF, TM, AO, TB).

Writing - Review & Editing. Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision — including pre-or post publication stages (AO, TB, ABH, TM, LB, THH, CF).

Visualization. Preparation, creation and/or presentation of the published work, specifically visualization/data presentation (TH, CF, TM, AO).

Supervision. Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team (TH, CF, AO, TM, TB).

Project administration. Management and coordination responsibility for the research activity planning and execution (TH, CF, TM).

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