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ORIGINAL ARTICLE

Antimicrobial efficacy of preoperative skin antisepsis and clonal relationship to postantiseptic skin-and-wound flora in patients undergoing clean orthopedic surgery

G. Daeschlein[1] · M. Napp[2] · F. Layer[3] · S. von Podewils[1] · H. Haase[1] · R. Spitzmueller[2] · O. Assadian[4] · R. Kasch[5] · G. Werner[3] · M. Jünger[1] · P. Hinz[2] · A. Ekkernkamp[2]

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Abstract Nosocomial surgical site infections (SSI) are still important complications in surgery. The underlying mechanisms are not fully understood. The aim of this study was to elucidate the possible role of skin flora surviving preoperative antisepsis as a possible cause of SSI. We conducted a two-phase prospective clinical trial in patients undergoing clean orthopedic surgery at a university trauma center in northern Germany. Quantitative swab samples were taken from pre- and postantiseptic skin and, additionally, from the wound base, wound margin, and the suture of 137 patients. Seventy-four patients during phase I and 63 during phase II were investigated. Microbial growth, species spectrum, and antibiotic susceptibility were analyzed. In phase two, the clonal relationship of strains was additionally analyzed. 18.0 % of the swab samples were positive for bacterial growth in the wound base, 24.5 % in the margin, and 27.3 % in the suture. Only 65.5 % of patients showed a 100 % reduction of the skin flora after antisepsis. The microbial spectrum in all postantiseptic samples was dominated by coagulase-negative staphylococci (CoNS). Clonally related staphylococci were detected in ten patients [nine CoNS, one methicillin-susceptible *Staphylococcus aureus* (MSSA)]. Six of ten patients were suspected of having transmitted identical clones from skin flora into the wound. Ethanol-based antisepsis results in unexpected high levels of skin flora, which can be transmitted into the wound during surgery causing yet unexplained SSI. Keeping with the concept of zero tolerance, further studies are needed in order to understand the origin of this flora to allow further reduction of SSI.

G. Daeschlein and M. Napp contributed equally to this work.

✉ G. Daeschlein
georg.daeschlein@uni-greifswald.de

[1] Department of Dermatology, University Medicine, Ernst-Moritz-Arndt University Greifswald, Sauerbruchstr., 17475 Greifswald, Germany

[2] Department of Trauma and Orthopedic Surgery, Clinic of Surgery, University Medicine, Ernst-Moritz-Arndt University Greifswald, Sauerbruchstr., 17475 Greifswald, Germany

[3] National Reference Center for Staphylococci and Enterococci, Division of Nosocomial Pathogens and Antibiotic Resistances, Department of Infectious Diseases, Robert Koch Institute, Wernigerode Branch, Wernigerode, Germany

[4] Clinical Institute of Hospital Hygiene, Medical University of Vienna, Vienna, Austria

[5] Department of Orthopaedics and Orthopaedic Surgery, University Medicine, Ernst-Moritz-Arndt University Greifswald, Sauerbruchstr., 17475 Greifswald, Germany

Introduction

Preoperative skin antisepsis (PSA) undoubtedly plays a crucial role in infection prevention by decreasing "endogenous" skin flora as a risk factor for surgical site infections (SSI) [1–3]. New preventive measures targeting the elimination or at least significant reduction of this flora by the use of new and potent antiseptics are currently being developed [4].

Following decades of continuously improving surgical techniques, accompanied by considerable efforts in hospital hygiene, it seems that the remaining "baseline level" of SSI represents an insurmountable obstacle to complete avoidance of SSI, which are still the third most frequently reported type of nosocomial infection [5]. Although such a baseline level of SSI per se may be more or less accepted in the case of low-risk situations, infection rates for certain currently increasing high-risk groups (i.e., immune-suppressed patients) and patients undergoing deep implant and bone surgery (i.e., hip replacement) may be dramatic, not to mention the potential collateral effect of pathogen transmission alone [6]. Additionally, SSI

may increase the costs of surgery by two to five times [7]. Finally, the question arises as to whether complete SSI prevention in clean surgery is realistic.

However, the problem of incomplete infection control practices cannot be faced as long as the underlying mechanisms of infection development are not fully known. Given that bacteria act as causative agents of SSI, their presence in situ may be due to different factors, e.g., insufficient preoperative skin antisepsis and/or environmental contamination [8–10].

In order to close the gap in preventive measures, the origin of wound flora during clean surgery as an etiologic event in infection development must be discovered. Therefore, the amount and composition of the skin flora before and after antisepsis, together with their clonal relationship (including wound flora), were analyzed in our study.

Materials and methods

Study design

This study was designed as a prospective monocentric clinical trial with two phases. In total, 137 patients (65 female, 72 male, mean age: 52±20 years; range: 18–96 years) were recruited, with 74 in phase I and 63 in phase II, with 4 years in between (139 surgical operations). During phase I, the amount, spectrum, composition, and antibiotic susceptibility of the encountered microbial species were studied. During phase II, the clonal relationship between pre- and postantiseptic flora on skin and in wounds (wound base, margin, and suture) was also analyzed. Follow-up was performed for 12 months; assessment of SSI (A1–A3) followed the Centers for Disease Control and Prevention (CDC) definitions [11] and was observed by the attending surgeon.

Patients were included if they underwent surgery for trauma of the spine (skin with a high density of sebaceous glands) or at the extremities (all category 1 procedures=clean surgery [11, 12]). Patients were excluded if they had infected or contaminated wounds, refused to participate, or were under the age of 18 years. The study was approved by the ethics committee of the Ernst Moritz Arndt University of Greifswald (registration no. BB 006/13). All patients gave informed consent to participate in the study.

Surgical procedures

In all cases, surgery was performed in the same surgical unit with a laminar air flow ventilation system (ceiling area 3.20×2.40 m). PSA was performed using a product based on propan-2-ol (70 % v/v; "Poly-Alkohol Antisepticum", Antiseptica, Pulheim, Germany) for an exposure of 4 min (normal skin, extremities) or 6 min (sebaceous skin, trunk). The antiseptic was applied three times as "surgical paint" in concentric circles extending from the incision site to the periphery using friction following the recommendations of the CDC and the Association of Operating Room Nurses (AORN) [11, 13]. The tool used was a sterile dressing forceps with an attached sterile gauze swab. The three swabs were discarded once the periphery had been reached. PSA was considered completed when the solution had thoroughly dried. All patients received perioperative antibiotic prophylaxis using one injection of 1.5 g cefuroxime i.v. administered 30 min prior to skin incision. The mean operation duration for spine surgery was 62.0±24.9 min and for surgery of extremities 75.3±42.5 min.

Table 1 summarizes the kind and frequencies of surgical interventions and the corresponding mean operation times. The most frequently conducted operation was osteosynthesis of the lower leg.

Microbiological sampling

An area of 10×10 cm in the center of the incision field was defined using a sterile, single-use stencil and marking its position with a sterile skin pen. Two swabs were taken from this area by the same trained investigator/surgeon (MN). The first swab (S1) was taken directly before PSA and the second (S2) after PSA immediately before incision by following the

Table 1 Kind and number of surgical procedures and mean operation duration

Procedure conducted	*n*	Mean operation duration (min)
Hip prosthesis	7	93.7±13.9
Knee prosthesis	2	104.0±32.5
Shoulder prosthesis	5	104.0±64.0
Osteosynthesis pectoral girdle	6	60.0±31.1
Osteosynthesis upper arm	9	102.8±27.2
Osteosynthesis forearm	18	64.6±24.8
Osteosynthesis spine	9	62.0±24.9
Pelvic osteosynthesis	3	108.0±61.5
Osteosynthesis thigh	5	64.4±46.7
Osteosynthesis knee	2	83.0±9.9
Osteosynthesis lower leg	33	83.8±54.9
Osteosynthesis foot	9	53.1±42.4
Soft-tissue surgery spine	1	26.0
Ligament reconstruction shoulder	3	85.0±10.4
Ligament reconstruction of the elbow	4	26.3±11.6
Ligament reconstruction knee	19	68.2±34.4
Open Achilles tendon rupture repair	3	38.7±3.2
Kind of operation unknown	1	121.0
Total	139	74.4±41.6

quantitative modified Levine technique [14]. Microbiological sampling of the wound was performed similar to the methods described by Benediktsdóttir and Hambraeus [15]: the wound base (S3) was sampled by carefully moving the swab into the deepest part of the wound with rotating movements over the whole length of the wound; the wound margin (S4) was sampled by swabbing both inner margins about 1 cm from the upper edge and touching stripe-like areas of the corium and the subcutis. The suture (S5) was sampled by swabbing along the border of both epidermal layers over the whole length of the wound (100 % epicutaneous suture).

Microbiological tests

Directly after sampling, swabs were placed into a sterile tube with transport medium (Amies medium) and immediately processed in the microbiology laboratory. Each swab was vortexed for 30 s in 3 ml of sterile saline (0.9 %) diluted with inhibitor [(NaClPeptone+LTHTh, Haipha GmbH, Eppelheim, Germany) the appropriate concentration was evaluated in prior experiments, data not shown), and 100 μL of the suspension was streaked onto Columbia agar (5 % sheep blood; Oxoid, Wedel, Germany) and incubated for 48 h at 36 °C. Grown colonies were counted and differentiated, then their susceptibility was tested using the VITEK® 2 Compact system (bioMérieux Deutschland GmbH, Nürtingen, Germany). The microbial growth was given as the number of colony-forming units (cfu) per swab of skin surface (according to an area of 10×10 cm for S1 and 2) and cfu per wound swab (samples S3–S5). All tests were carried out in accordance with current national specifications established by professional associations [16]. The reduction factor (RF) of antisepsis was calculated as follows: RF=Log(total amount of cfu before antisepsis) − Log(total amount of cfu after antisepsis).

PFGE and *spa* typing

For staphylococci which presented with similar cultural colony morphology and identical antimicrobial susceptibilities (minimal inhibition concentration result from the VITEK system), clonal identity was suspected and tested by *Sma*I macrorestriction in pulsed-field gel electrophoresis (PFGE) and subsequent fragment pattern analysis using BioNumerics 7.0 (Applied Maths, Belgium; for a detailed technical description, see [17]). Additionally, *spa* typing was carried out by amplifying the polymorphic X region of the *spa* gene (using primers spa-1113f and spa-1514r); sequencing was performed as described previously [18]. Ridom StaphType software version 1.4 (Ridom GmbH) was used for analysis and SpaServer (http://www.spaserver.ridom.de) was used for synchronization [17, 18].

Statistics

Mean cfu before and after antisepsis were compared using the two-sample *t*-test (α=0.5). Additionally, corresponding to differences between the two phases, the reduction factors (RF, decadic logstep reduction) of cfu before and after antisepsis were compared using this test. The free software package R (R Development Core Team, 2009) was used for the statistical analyses.

Results

Microbial growth and antiseptic efficacy

In phase I, 172.1±432.4 cfu/swab were counted before and 22.7±56.9 cfu/swab after antisepsis, corresponding to an RF of 1.4. In phase II, 151.4±260.1 cfu/swab were found before and 64.5±137.2 cfu/swab after antisepsis, corresponding to an RF of 0.8. Pooling both phases, we calculated an RF of 1.0, corresponding to the 162.8±363.7 cfu/swab before and 44.5±107.6 cfu/swab found after antisepsis. The difference between the microbial load (cfu) before and after antisepsis (both phases) was significant (p=0.00002).

65.5 % (n=91) of the patients showed a 100 % reduction of the initial skin flora (Fig. 1). When the two phases were differentiated, these values were 68.9 % (n=51) in phase I and 61.5 % (n=40) in phase II (Fig. 2). The patients with bacterial growth after antisepsis (34 %) showed 1 to 612.5 cfu per sample (mean total amount=44.5±107.6 cfu/swab).

2.9 % (n=4) of all skin samples (both phases) showed a bacterial load which was not found before skin antisepsis. This flora was defined as “neo burden” (Fig. 1). In 7.9 % (n=11) of skin samples, the bacterial load which was encountered before antisepsis showed increasing amounts during intervention (Fig. 1).

Overall, 64 (46.0 %) patients showed microbial growth in at least one wound area during surgery. Of these patients, 39 (28.1 %) showed microbial growth in only one, 18 (12.9 %) in two, and 7 (5.0 %) in all three wound areas. In 42 % (n=27) of the cases, the finding of at least one positive wound sample was related to positive skin samples after PSA.

In the wound base, 18.0 % of swab samples were positive for bacterial growth, as were 24.5 % in the wound margin and 27.3 % in the wound suture.

In nine sample swabs, methicillin-susceptible *Staphylococcus aureus* (MSSA) was cultured before PSA on skin. In one of these nine sample swabs, MSSA was also cultured from the wound base and wound margin during surgery.

Table 2 shows the percental distribution of the encountered bacterial species before and after antisepsis. By far, coagulase-negative staphylococci (CoNS) were the predominating

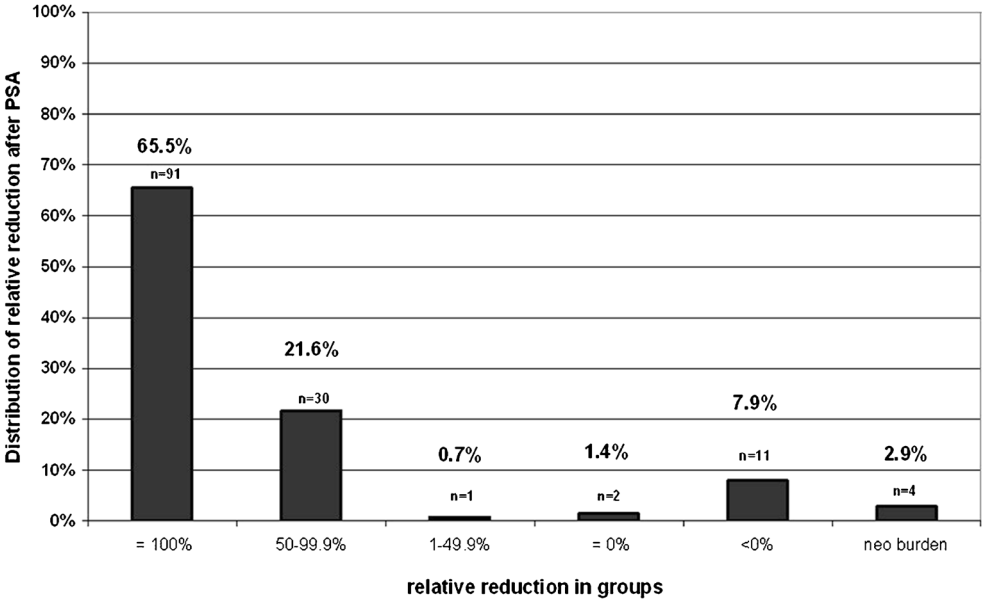


Fig. 1 Distribution of relative reduction (%) of bacteria sampled on skin after preoperative skin antisepsis (PSA; both phases)

bacteria before PSA with 192 isolates in 126 skin samples (90.6 %), followed by *Micrococcus* spp. with 59 isolates in 58 samples (41.7 %). After antisepsis, CoNS were still the predominating bacteria on skin with 39 isolates in 29 skin samples (20.9 %), followed by aerobic spore-forming bacilli with 15 isolates in 15 skin samples (10.8 %). In the wound base, wound margin, and suture, CoNS were also the most frequent bacteria with 23 isolates in 20 (14.4 %) samples from the wound base, 31 isolates in 28 (20.1 %) margin samples, and 29 isolates in 26 (18.7 %) suture samples. In wound base, margin, and suture, CoNS were followed by *Micrococcus* spp. with 3 isolates in 3 (2.2 %) samples from wound the base, 5 isolates in 5 (3.6 %) margin samples, and 8 isolates in 8 (5.8 %) suture samples. By calculating the mean number of cfu per recovered species, CoNS showed the highest number of all species on skin both before antisepsis with 92.5 cfu± 264.5 cfu and after antisepsis with 44.7 cfu±76.4 cfu.

Aside from MSSA, three further hospital pathogens were detected on preoperative skin (S1) in small amounts (one isolate each): *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Sphingomonas paucimobilis*, of which no multidrug-resistant isolate was proven by susceptibility testing. Eighteen of all

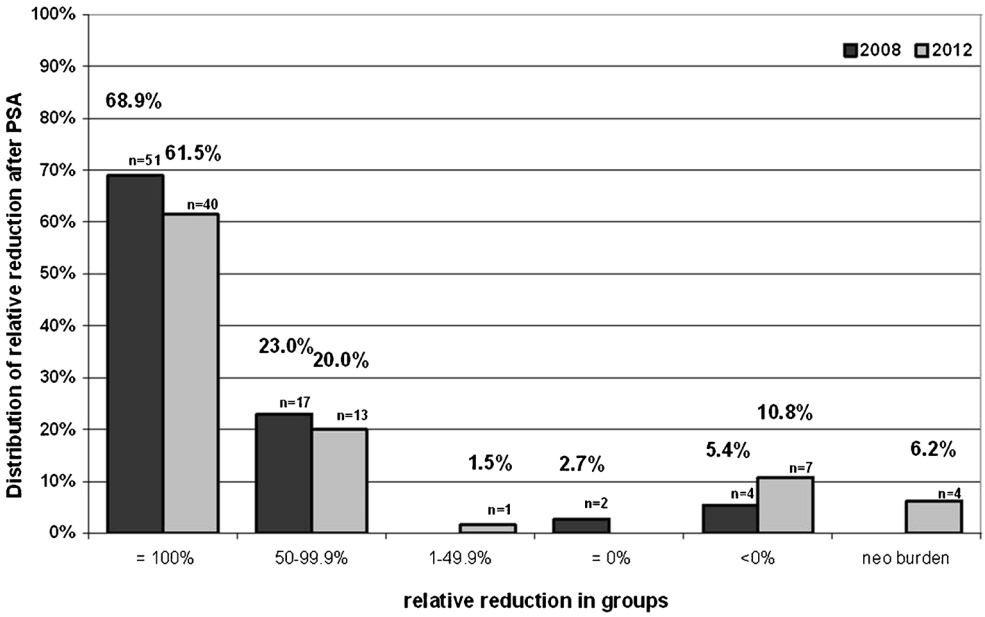


Fig. 2 Distribution of relative reduction (%) of bacteria sampled on skin after PSA differentiated by phase

Table 2 Distribution (absolute and percentage) of the encountered bacterial species in total, before, and after antisepsis (on skin)

	Before PSA		After PSA	
	n isolates (%)	Mean cfu/ isolate (SD)	*n* isolates (%)	Mean cfu/ isolate (SD)
Total	322 (100.0)	68.2 (±211.0)	73 (100.0)	29.2 (±60.2)
CoNS*	192 (59.6)	92.5 (±264.5)	39 (53.4)	44.7 (±76.4)
Micrococcus spp.	59 (18.3)	28.6 (±61.7)	12 (16.4)	8.5 (±1 7.0)
Aerobic spore-forming bacilli	33 (10.2)	26.7 (±76.3)	15 (20.5)	7.1 (±11.2)
Streptococcus spp.	12 (3.7)	84.4 (±106.5)	5 (6.8)	35.0 (±52.1)
Corynebacterium spp.	11 (3.4)	30.0 (±83.0)	1 (1.4)	1.0
MSSA	9 (2.8)	25.7 (±36.3)	0 (0.0)	–
Candida spp.	2 (0.6)	1.0 (±0.0)	0 (0.0)	–
Proteus mirabilis	1 (0.3)	30.0	0 (0.0)	–
Pseudomonas aeruginosa	1 (0.3)	15.0	0 (0.0)	–
Sphingomonas paucimobilis	1 (0.3)	10.0	0 (0.0)	–
Enterococcus spp.	0 (0.0)	–	1 (1.4)	4.0
Not specified	1 (0.3)	3.0	0 (0.0)	–

*With three isolates of *Staphylococcus epidermidis* (MRSE) and 15 further CoNS isolates showing oxacillin resistance (one *Staphylococcus haemolyticus* with oxacillin resistance was detected before and after PSA)

detected CoNS isolates showed oxacillin resistance. The respective numbers of the other species are shown in Table 2. The distribution of resistant and susceptible CoNS isolates to different antibiotics is shown in Table 3.

Table 3 Relative distribution (%) of resistant/intermediate or susceptible isolates of coagulase-negative staphylococci (CoNS) with respect to each tested antibiotic

	% R/I	% S
Fosfomycin	61.9 %	38.1 %
Erythromycin	60.3 %	39.7 %
Benzylpenicillin	54.0 %	46.0 %
Clindamycin	38.1 %	61.9 %
Oxacillin	28.6 %	71.4 %
Tetracycline	25.4 %	74.6 %
Tobramycin	22.2 %	77.8 %
Levofloxacin	12.7 %	87.3 %
Fusidic acid	9.5 %	90.5 %
Gentamicin	7.9 %	92.1 %
Trimethoprim/sulfamethoxazole	7.9 %	92.1 %
Moxifloxacin	3.2 %	96.8 %
Rifampicin	3.2 %	96.8 %
Nitrofurantoin	1.6 %	98.4 %
Linezolid	0.0 %	100.0 %
Teicoplanin	0.0 %	100.0 %
Tigecycline	0.0 %	100.0 %
Vancomycin	0.0 %	100.0 %
Mupirocin	0.0 %	100.0 %

Clonal relationship in phase II

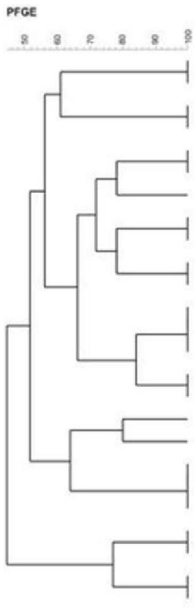
The tests for clonal identity resulted in suspected identity of staphylococci in ten patients, as proven by PFGE. Nine of them (14.3 % of the 63 patients) were CoNS and one MSSA (Fig. 3), with eight of these patients showing double and two triple positivity in their wounds.

Among the patients with two areas showing the same clone, three patients had *S. epidermidis*, three *S. hominis*, one *S. lugdunensis*, and another one *S. capitis*. One of the two patients showing three identical clones had *S. haemolyticus*, the second MSSA.

In five of the ten patients with clonally related species, the preantiseptic skin flora (S1) was clonally involved in the postantiseptic flora (S2) (including wounds S3–S5). In these five cases, *S. capitis*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and MSSA were involved (Fig. 3) and found in preantiseptic skin (S1), S2 (patient 2 with *S. capitis*), S3 (patient 2 with *S. hominis*), S4 (patient 5 with *S. epidermidis*), S4 and S5 (patient 6 with *S. haemolyticus*), and one patient showing *S. aureus* in S3 and S4 (patient 8).

In five patients, identical clones were found in two different sample sites without preantiseptic skin (S1) involvement. They involved one patient with *S. hominis* in S2 and S3 (patient 9) and a second in S3 and S5 (patient 10), one with *S. lugdunensis* in S3 and S4 (patient 1), one with *S. epidermidis* in S2 and S3 (patient 3), and another in S3 and S4 (patient 4) (Table 4 and Fig. 3).

All remaining positive microbial growth did not show any clonal relation to other isolates recovered from any of the test



patient	sample site	species	germ no.	resistance phenotype	id	spa-type
1	S3	S. lugdunensis	1	PEN, ERY, CLI	1 and 2 = id	
1	S4	S. lugdunensis	2			
2	S1	S. capitis	3	PHO	3 and 4 = id	
2	S2	S. capitis	4	PEN, PHO		
3	S2	S. epidermidis	5	SUSCEPTIBLE	5 and 6 = id	
3	S3	S. epidermidis	6			
4	S1	S. epidermidis	7	SUSCEPTIBLE		
5	S1	S. epidermidis	8	PEN, ERY	8 and 9 = id	
5	S4	S. epidermidis	9			
4	S3	S. epidermidis	10	PEN, TPL	10 and 11 = id	
4	S4	S. epidermidis	11			
6	S1	S. hemolyticus	12	PEN, OXA, GEN, ERY, TET i, CIP, SXT, PHO, MFL (i), OxaSu	12, 13 and 14 = id	
6	S4	S. hemolyticus	13			
6	S5	S. hemolyticus	14			
2	S1	S. hominis	15	PHO	15 and 16 = id	
2	S3	S. hominis	16			
7	S1	S. aureus	17	SUSCEPTIBLE		t008
2	S1	S. aureus	18	SUSCEPTIBLE		t091
8	S1	S. aureus	19	SUSCEPTIBLE		t728
8	S3	S. aureus	20	PEN	19, 20 and 21 = id	t728
8	S4	S. aureus	21	PEN		t728
9	S2	S. hominis	22	PHO	22 and 23 = id	
9	S3	S. hominis	23			
10	S3	S. hominis	24	ERY, TET, PHO	24 and 25 = id	
10	S5	S. hominis	25			

Fig. 3 Clonal relationship in six different staphylococcal species (25 strains) of ten patients from phase II, proven by pulsed-field gel electrophoresis (PFGE) analysis

areas (data not shown), thus being obviously caused by sporadic environmental contamination.

spa typing of isolates of *S. aureus* as performed at the RKI revealed identical clones in patient 8 in the S1, S3, and S4 samples with *spa* type t728, thus confirming PFGE typing (Table 4 and Fig. 3). Previously, this *spa* type had only been described in patients suffering from bacteremia in Norway and frequently in healthy food handlers in Bosnia and Herzegovina [19, 20].

Clinical course of patients

One patient developed a low-grade infection of a total knee replacement. To date, she has refused revision of the implant.

Table 4 Identical clone distribution and sample sites. Every line delineates a specific clone of CoNS or MSSA (**bold**)

patient number	S1	S2	S3	S4	S5
1			+	+	
2	+	+			
2	+		+		
3		+	+		
4			+	+	
5	+			+	
6	+			+	+
8	+		+	+	
9		+	+		
10			+		+

No other patient showed SSI or healing disturbance within 12 months after surgery.

Summary of results

Ethanol-based routine antisepsis in trauma surgery enabled a 100 % reduction of the detectable preoperative skin flora in only 65.5 % of operations. Moreover, in 8 % of the operations, the bacterial load after PSA was higher than before. In every sample site, CoNS were the predominant bacterial species.

In 10 (15.4 %) operations, clonally related staphylococci were detected, 6 (60 %) of these with apparent transmission of pre- or postantiseptic skin flora into the wound. The clonal results also revealed an apparent environmental wound contamination.

Discussion

Although the introduction of the aseptic operative technique and PSA by Lister 150 years ago and modern infection control measures such as the multibarrier strategy could not completely avoid SSI, they have reduced them to a "basic level" [11, 12]. The causes of this baseline level of SSI seemingly deriving from microbes transmitted into the wound are not fully understood yet. Therefore, potential bacterial sources should be addressed. However, PSA may play a more important role in terms of SSI prevention in clean surgery than in non-clean surgery, as there may be a lower risk of "internal" contamination. Skin flora, especially staphylococci, are regularly found in deep infections of orthopedic surgery, e.g., prosthetic joint

infections [6, 21]. Three main causative scenarios may be defined:

- Low antimicrobial efficacy (after contact with the antiseptic), leaving vital microbes in the skin which easily move into the wound.
- Failure to contact pathogens with the antiseptic, which does not reach bacteria in deeper skin (corneal cell layers) areas, glands, and follicles.
- Contamination of the wound and/or skin by environmental microbes which originate from non-disinfected skin or wound areas and/or from other environmental contamination sites. Finally, all these microbes can be transmitted via contaminated air or aerosols into the wound. This scenario also includes contamination either by surface cross-contamination via external glove surfaces or by pathogens from the glove fluid entering the wound after glove perforation [22, 23].

These examples may explain the occurrence of SSI despite accurate antiseptic and perioperative preventive measures, because, in fact, all these scenarios may allow active (secretions, flow, diffusion, regrowth) or passive (transmitted via gloves, instruments, or foreign bodies) transmission of nearby pathogens into the surgical wound, where they can multiply and, ultimately, produce SSI or healing disturbance. Moreover, it is possible that two or all three of these scenarios work together.

In a study regarding hand hygiene, 66 % of healthy participants (n=60) still had detectable bacteria after performing an alcohol-based hand rub [24]. However, in contrast to hand hygiene, no data regarding the efficacy of alcohol-based preoperative skin antisepsis without other ingredients are available in the literature. Nevertheless, the same mechanisms involved in hygienic hand antisepsis can be suggested for alcohol-based PSA, providing a background against which our data can, at least in part, be interpreted.

With other surgical preparation solutions, similar survival rates of skin flora were found by Ostrander et al. on intact skin of the lower leg: PSA with 3.0 % chloroxylenol (TechniCare®), 0.7 % iodine and 74 % isopropyl alcohol (Duraprep®), and 2 % chlorhexidine gluconate and 70 % isopropyl alcohol (Chloraprep®) allowed bacterial growth in 35 %, 23 %, and 10 % (n=125) of the cases, respectively, but species differentiation was not reported [25].

We repeatedly found unexpectedly large amounts of bacteria on both the skin and in the wounds in both phases. This finding does not seem related to seasonal or interventional conditions and, therefore, can be seen as realistic and relevant support for the origin of SSI in surgical practice. A distribution of microbial populations similar to that found in this study was detected by Edmiston et al. in the operating room regarding airborne transmission [10].

The first wound contamination scenario (poor antiseptic efficacy) mentioned above seems quite unrealistic, because no doubt currently exists about the proven efficacy of common antiseptics. Additionally, the worst-case scenario of masses of microbes possibly overwhelming antiseptic activity (i.e., abscess) in the presence of large amounts of protein (secretions) and, thus, inhibiting the antiseptic activity can be excluded in our patients, as they had undergone clean antiseptic surgery.

The second scenario is far more realistic; even supposing adequate application, the failure of the antiseptic to contact pathogens may be explained by bacteria escaping microbiocidal attack in deeper skin niches, e.g., in glands, follicles, and deep-seated corneal spaces not reached by AS liquids, as shown for alcohol-based PSA [26]. These commensals are difficult to remove completely, in contrast to the transient organisms at the skin's surface, and are suspected of being responsible for the regrowth of skin flora after applying skin antisepsis on the skin surface [27].

In our patients, a clonal relationship of wound flora to skin flora, i.e., to postantiseptic flora in 2 of 10 patients and to preantiseptic skin flora in 4 of 10 patients, was seen. In contrast, one patient showed a direct relationship between postantiseptic and preantiseptic skin flora without wound contamination (patient 2). These data support the provenience of wound flora, at least in part, by skin flora escaping the antiseptic attack as described above.

Compatible with the third scenario (environmental contamination), the clonal results from five patients also show wound staphylococci that are not related to preantiseptic flora, thus supposing an environmental provenience of this flora. The probability of glove breach as a cause of environmental contamination in our study is low, as the surgical staff regularly practiced double gloving, and previous internal quality control investigations revealed glove breach in 5–8 % of surgical procedures (data with the authors). Environmental contamination of wounds has been well documented in previous studies, but despite the implementation of laminar air flow ventilation, cross-contamination with skin flora originating from the patient her-/himself or the staff has been described [10, 28]. Most of the studies show environmental transmission of staphylococci [8–10, 28]. Ultimately, most kinds of contamination in the operating room may be supposed to originate from the skin of the patient or the staff [8–10, 28].

Our data underline the importance of PSA as a crucial part of surgical hygiene. The microbiological analysis, together with clonal investigation, strongly suggests two equally important wound contamination scenarios: first, hidden skin flora escaping contact with the antiseptic and second, environmental flora, both of which may lead to pathogen transmission into the wound and infection development.

As the two phases 4 years apart showed nearly identical contaminations, it can be assumed that this contamination

level is realistic at least for this kind of surgery, and not only in our hospital. Despite the unexpectedly high rate of microbial growth after PSA on skin (35 %), together with concomitant wound positivity (46.0 % of wounds showing growth in at least one of the three sampled wound areas), our SSI rates (0.73 %) did not surpass the level referenced for clean surgery (<2 %) [12]. This may be attributable to the prophylactic antibiotics. As CoNS show frequent resistance to cefuroxime (deducted from 28.6 % of CoNS with oxacillin resistance in our data), we can assume that the single injection would not reliably be of any use. The present data show that trimethoprim/sulfamethoxazole may be most promising, with 92 % susceptibility of CoNS to be expected. The routine use of the other antibiotics, to which microbes are far more susceptible (see Table 3), does not seem suitable, for instance due to risk of selection for resistant strains (MRGN by gyrase inhibitors) or the possible development of resistance in enterococci (VRE) by vancomycin [29].

In any case, such quantifiable levels of bacterial burden justify the attempt to further reduce this microbial load and, thus, eliminate one possible precondition for SSI. Further studies must be undertaken in order to prove this hypothesis. Achieving such a reduction by "better" antisepsis is controversial, since it is acknowledged that conventional antiseptics are unable to reach subsurface microbes [4, 30]. The question arises as to whether to modify the preoperative antiseptic strategy, e.g., by repeatedly applying liquid antiseptic to the surgical site, or by looking for new antiseptics which can bypass the activity gap in deeper skin. Cold atmospheric plasma (CAP), which is a completely different approach to antimicrobial treatment, has been shown to act not only against surface flora but also against the follicle flora [30]. Further work must be done to confirm these results, but, in the future, the application of CAP to the wound during surgery may become a new strategy in infection prevention.

In addition to the staff properly performing good surgical and general hygiene, the prevention of environmental contamination may be achieved by the use of surgical gloves with antimicrobial properties, e.g., antimicrobial coating of the external or internal glove surface or adding to the glove a layer with active antiseptic compounds, thus avoiding microbial contamination by glove fluid in case of glove breach [11, 13, 22, 23].

Conclusion

The current study found unexpectedly high amounts of microbial contamination in surgical wounds, apparently deriving to equal extents from the skin, despite properly performed routine skin antisepsis, and from environmental contamination.

The well-documented importance of staphylococci in surgical site infections (SSI) was confirmed by clonal analysis as well as our qualitative and quantitative results, with this species predominating in all samples. Therefore, staphylococci remain the main target of anti-infective and preventive measures.

Compliance with Ethical Standards All authors disclose any potential conflict of interest (e.g., employment, consulting fees, research contracts, stock ownership, patent licenses, honoraria, advisory affiliations, etc.).

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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