Evaluation of a multilocus sequence typing system for *Staphylococcus epidermidis*

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Staphylococcus epidermidis is a significant cause of nosocomial disease. However, the taxonomy of this pathogen, particularly at subspecies level, is unclear. A multilocus sequence typing (MLST) scheme has therefore been investigated as a tool to elucidate taxonomic relationships within this group, based on genetic relatedness. DNA sequences for internal fragments of seven housekeeping genes were compared in 47 geographically and temporally diverse *S. epidermidis* isolates that were obtained from clinical infections. Twenty-three different allelic profiles were detected; 17 of these were represented by single strains and the largest profile group contained 17 isolates. Diversity of the same collection of isolates was investigated by using PFGE of *Sma*I-digested genomic DNA to test the discrimination and validity of the MLST approach. Isolates within the largest profile group were resolved into four distinct PFGE clusters on the basis of their *Sma*I digest patterns. Isolates within other profile groups that contained multiple isolates had matching PFGE *Sma*I patterns within each group. It appears that MLST is an effective method for grouping *S. epidermidis* strains at the subspecies level; however, it is not as discriminatory as it has been for other species for which MLST schemes have been established and, used alone, would not be a useful method for epidemiological studies. In addition, it was demonstrated that this method was effective for confirming the identity of *S. epidermidis* CoNS (coagulase-negative) isolates.

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INTRODUCTION

The genus *Staphylococcus* is a member of the family *Micrococcaceae* and consists of over 40 species and subspecies at the time of writing. Staphylococci are differentiated by their ability to produce coagulase into the coagulase-positive (*Staphylococcus aureus*) and coagulase-negative (CoNS) groups (Lina *et al.*, 2000). Within the CoNS group, *Staphylococcus epidermidis* is the species that is associated most commonly with disease. The Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) study reported that of 5000 bloodstream infections from 41 US hospitals that were assessed during the 1995–1996 reporting

period, CoNS were the major cause (30.5%); when 787 of these isolates were speciated, 602 (78%) were identified as *S. epidermidis* (Marshall *et al.*, 1998). Despite the clinical relevance of this organism, relatively little is known about its taxonomy at the subspecies level. This is partly because once a staphylococcal isolate has been assigned to the CoNS group, it is often not identified to the species level. Understanding of the diversity within and relationships among pathogenic taxa is an important prerequisite to meaningful taxonomic classification, accurate identification and pathogen detection and for epidemiology studies. Several taxonomic studies of *S. epidermidis* have been undertaken by using molecular techniques; these include PFGE (Linhardt *et al.*, 1992; Bannerman *et al.*, 1997); DNA–DNA hybridization (Villari *et al.*, 2000); repetitive element sequence-based PCR (Hu & Totake, 1997); sequence analysis of pathogenicity islands (Nahvi *et al.*, 2001) and specific genes (Deplano *et al.*, 1997); randomly amplified polymorphic DNA (RAPD) (Bogado *et al.*, 2002; Dautle *et al.*, 2002); PCR-amplified fragment length polymorphism (AFLP) (Sloos *et al.*, 1998); ribotyping (Geary *et al.*, 1997); plasmid profiles and phenotypic techniques, including anti-

Abbreviations: CoNS, coagulase-negative staphylococci; MLST, multilocus sequence typing; NICU, neonatal intensive-care unit; RAPD, randomly amplified polymorphic DNA; ST, sequence type.

The GenBank/EMBL/DDBJ accession numbers for the sequences generated by this work are as follows: yqil1–yqil10, AY163262–AY163271; tpi1–tpi9, AY163272–AY163280; pta1–pta6, AY163281–AY163286; gmk1–gmk7, AY163287–AY163293; glpF1–glpF9, AY163294–AY163302; aroE1–aroE10, AY163303–AY163312; hsp1–hsp7, AY323453–AY323459.

biograms (Sloos *et al.*, 1998); and API identification strips (Sloos *et al.*, 2000). However, these studies are often laboratory-specific and not polyphasic, making it difficult to correlate results and to assess true diversity within this clinically important bacterial species.

PFGE is the favoured method for *S. epidermidis* epidemiological studies and is useful for tracking specific outbreaks. Its utility is based on direct comparison of RFLPs of the entire genome when restricted with a rare-cutting enzyme. Given that mutations can occur at a relatively high frequency, including in the rare-cutting restriction sites, a single genetic alteration can alter three bands in a PFGE pattern (Tenover *et al.*, 1995). Increased changes over time limit the use of PFGE analysis for taxonomic studies and in long-term or multi-centre studies. This method also has the drawback of being extremely time-consuming and subjective in interpretation and it is difficult to record PFGE patterns and their identification. As with the other molecular methods listed above, PFGE does not transfer well between laboratories, as demonstrated by van Belkum *et al.* (1998), who conducted a large interlaboratory study testing the reproducibility of PFGE for staphylococci. Although the method is robust, it is subject to operator variation.

An alternative molecular classification method is multilocus sequence typing (MLST), a sequence-based method first described by Maiden *et al.* (1998), where it was used to delimit strain associations and clonal groupings within a reference set of *Neisseria meningitidis* isolates. It was developed as a robust typing method that measures sequence divergence of several genes that are subject to slow rates of change, thus making it suitable for long-term epidemiological studies. Furthermore, as data are in the form of sequences, they can be catalogued easily and compared readily among laboratories. This method has since been demonstrated to be an effective method for the study of taxonomic and genetic diversity of other microbial pathogens, including *Streptococcus pneumoniae* (Enright & Spratt, 1998), *Staphylococcus aureus* (Enright *et al.*, 2000), *Campylobacter jejuni* (Dingle *et al.*, 2001), *Salmonella* spp. (Kotetishvili *et al.*, 2002), *Candida albicans* (Bougnoux *et al.*, 2002) and *Enterococcus faecalis* (Nallapareddy *et al.*, 2002).

The aim of this study was to evaluate the use of an MLST-based scheme to study the diversity of a set of *S. epidermidis* isolates selected randomly from a collection of isolates that were obtained from geographically dispersed hospitals and associated with different disease states.

METHODS

Bacterial strains and culture conditions. Bacterial strains investigated in this study are listed in Table 1. *S. epidermidis* strains were of clinical origin and were isolated from various sites and geographical areas. Strains were initially received on tryptone soy agar (TSA) II+5% sheep blood agar (BBL; Becton Dickinson) and transferred subsequently to Microbank porous carrier beads (Pro-Lab Diagnostics) for long-term storage at -80°C . Cultures were grown by inoculating TSA II+5%

sheep blood agar plates directly with a carrier bead and incubating at 37°C overnight. A clonal isolate was picked to inoculate 3 ml tryptic soy broth (Difco) and incubated overnight at 37°C with shaking at 150 r.p.m.

Small-scale isolation of chromosomal DNA. Overnight cultures were centrifuged in 1.5 ml Eppendorf tubes at 14 000 r.p.m. for 5 min. Broth was decanted and pellets were resuspended in 500 μl resuspension buffer (25% sucrose, 10 mM Tris, pH 7.5). Suspensions were lysed with 20 μg lyphostatin ml^{-1} (Sigma-Aldrich) and were incubated at 37°C for 1 h. At the end of the incubation period, 250 μl 2% SDS was added to each tube and vortexed until the viscosity of the solution decreased noticeably. Phenol/chloroform/isoamyl alcohol solution (250 μl) (25:24:1, v/v) (Gibco/Invitrogen) was added. The mixture was vortexed for 30 s and centrifuged for 5 min at 14 000 r.p.m. The aqueous phase was removed and the precipitation steps were repeated until almost no interface remained; 0.1 vols 3 M NaOAc, pH 4.8, were added to each tube and mixed. One volume of 2-propanol was then added and the tube was mixed again. Tubes were left to incubate for 5 min at room temperature and then centrifuged at 14 000 r.p.m. for 15 min. Supernatant was decanted and tubes were allowed to dry upside-down on tissue. Pellets were resuspended in 50 μl sterile H_2O . Multiple ($5\times$) DNA preparations were performed on one isolate (*S. epidermidis* ATCC 35984) to evaluate the robustness of the technique.

MLST. Nucleotide sequences of seven *S. epidermidis* housekeeping genes, shikimate dehydrogenase, heat-shock protein, glycerol kinase, guanylate kinase, phosphate acetyltransferase, triphosphate isomerase and acetyl coenzyme A acetyltransferase, were obtained from GenBank and PCR primers were designed to amplify fragments that ranged from 491 to 620 bp in size (Table 2). Approximately 50 ng template bacterial DNA was PCR-amplified with each primer set by using a BD Advantage cDNA PCR kit (BD Clontech) according to the manufacturer's instructions and an Applied Biosystems GeneAmp 9700 thermocycler. An initial denaturation at 94°C for 1 min was followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 30 s and extension at 68°C for 4 min and a final extension at 68°C for 4 min. Amplified DNA was purified with a QIAquick PCR Purification kit (Qiagen) and both strands were sequenced by using BigDye fluorescent terminators (Applied Biosystems) and the amplification primers on an ABI 377 DNA Sequencer (36 cm gel for 7 h). Sequences were analysed by using Sequencer 3.1 (GeneCodes).

Sequences obtained were assigned allele numbers that were used to generate sequence types (STs) for each isolate. Allelic profiles were compared by using the simple matching coefficient and clustered by UPGMA (NTSYSpc; Exeter Publishing). BURST analysis was done by using software from the MLST site (<http://www.mlst.net>).

PFGE. Each isolate was genotyped by PFGE using the restriction enzyme *Sma*I (New England BioLabs) and performed according to methods described previously (Roberts *et al.*, 1998). Macrorestriction profiles were interpreted as described by Tenover *et al.* (1995).

Resistance to methicillin. MIC values of the isolates to methicillin were determined at their hospitals of origin by using standard procedures. Bacterial suspensions in water were adjusted to a McFarland standard of 0.5. *In vitro* susceptibility tests (MIC) were conducted by inoculating microwell susceptibility test cards; analysis was performed by the bioMérieux Vitek automated system. MIC values were determined according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Isolates with MIC values to oxacillin that were below 2 mg l^{-1} were considered to be susceptible to methicillin and those with MIC values above 2 mg l^{-1} were considered to be resistant. To confirm these results, chromosomal digests were probed for the presence of the *mecA* gene by using the method described by Kreiswirth *et al.* (1993).

Table 1. Staphylococci used in this investigation and corresponding STs, BURST groupings and PFGE profilesATCC, American Type Culture Collection; MB, Merck bacteria collection; MCL, Merck clinical collection; MRSA, methicillin-resistant *Staphylococcus aureus*; R, resistant; S, susceptible.

Organism (strain)	Source	Allelic profile							ST group	BURST group	PFGE profile	Methicillin resistance	mecA gene
		aroE	glpF	gmk	pta	tpi	yqiL	hsp					
Staphylococcus epidermidis:													
RP62A	Also known as ATCC 35984; catheter sepsis, Tennessee, USA (pre-1982)	1	1	1	1	1	1	1	1	S		S	+
MB2230	Also known as ATCC 12228	1	2	2	2	1	1	1	2	1	2-2	S	—
1	Urine, Lexington, KY, USA; MCL8616	1	2	2	2	1	1	1	2	1	2-1	R	+
2	Exudate right inguina, Sun City, AZ, USA; MCL8719	1	2	2	2	1	1	1	2	1	2-1	R	+
3	Left shoulder, Ft Myers, FL, USA; MCL8758	1	2	2	2	1	1	1	2	1	2-1	R	+
4	Pus, Kalafong, South Africa; MCL12366	1	2	2	2	1	1	1	2	1	2-1	R	+
5	Catheter, uterus, Pretoria, South Africa; MCL12370	1	2	2	2	1	1	1	2	1	2-1	S	+
6	Exudate, abdomen, Philadelphia, PA, USA; MCL12546	1	2	2	2	1	1	1	2	1	2-1	S	—
7	Peritoneum, Buffalo, NY, USA; MCL8225	1	2	2	2	1	1	1	2	1	2-2	R	+
8	Left ankle, Springfield, IL, USA; MCL8847	1	2	2	2	1	1	1	2	1	2-2	R	+
9	Pus, appendix, Parow, South Africa; MCL12296	1	2	2	2	1	1	1	2	1	2-2	R	+
10	Abdominal wound, Torrance, CA, USA; MCL8888	1	2	2	2	1	1	1	2	1	2-3	R	+
11	Urine, Kent, WA, USA; MCL8519	1	2	2	2	1	1	1	2	1	2-3	S	—
12	Blood, Los Angeles, CA, USA; MCL8321	1	2	2	2	1	1	1	2	1	2-4	R	+
13	Right foot, Wichita, KS, USA; MCL8568	1	2	2	2	1	1	1	2	1	2-4	R	+
14	Left foot, New Orleans, LA, USA; MCL8751	1	2	2	2	1	1	1	2	1	2-4	R	+
15	Blood, Providence, RI, USA; MCL10478	1	2	2	2	1	1	1	2	1	2-4	S	+
16	Right arm pus, Baltimore, MD, USA; MCL8952	1	2	2	2	1	1	1	2	1		R	+
17	Peritoneum, Los Angeles, CA, USA; MCL8192	1	2	6	2	1	1	1	3	1		R	+
18	Intra-abdominal, NYC, NY, USA; MCL8359	1	2	6	2	1	1	1	3	1		R	+
19	Pus, Pretoria, South Africa; MCL11771	1	2	6	2	1	1	1	3	1		S	+
20	Endometrium, Los Angeles, CA, USA; MCL12846	1	2	6	2	1	1	1	3	1		S	—

(continued overleaf)

Table 1. cont.

Organism (strain)	Source	Allelic profile							ST group	BURST group	PFGE profile	Methicillin resistance	mecA gene
		aroE	glpF	gmk	pta	tpi	yqiL	hsp					
21	Blood, NYC, NY, USA; MCL11615	1	2	2	2	7	1	1	4	1		R	+
22	Pus, wound, South Africa; MCL12307	1	2	2	2	7	1	1	4	1		R	+
23	Exudate, face, Brazil; MCL12000	2	3	3	3	2	2	2	5	S		R	—
24	Right arm, Springfield, IL, USA; MCL8827	2	3	3	3	2	2	2	5	S		S	—
25	Abdomen, Springfield, IL, USA; MCL8851	3	2	2	2	1	1	1	6	1		R	+
26	Incision, wound, Wilmington, DE, USA; MCL9430	1	2	2	1	1	1	1	7	1		R	+
27	Left arm, Sun City, AZ, USA; MCL9689	1	2	2	1	1	1	1	7	1		R	+
28	Wound, Orange, CA, USA; MCL10141	1	2	2	1	1	1	1	7	1		S	+
29	Intra-peritoneal pus, Buffalo, NY, USA; MCL9891	1	2	2	2	1	3	1	8	1		R	+
30	Right tibial wound, Honolulu, HA, USA; MCL8453	1	2	2	2	1	3	1	8	1		R	+
31	Right leg tissue, Wichita, KS, USA; MCL8809	1	2	1	1	3	1	1	9	S		S	—
32	Pus, gall bladder, Wilmington, DE, USA; MCL9423	1	2	2	1	1	10	1	10	1		R	+
33	Blood, Miraflores, Peru; MCL10484	9	4	1	1	5	4	4	11	S		S	+
34	Blood, Pretoria, South Africa; MCL12281	1	2	2	6	1	1	1	12	1		R	+
35	Pus, right leg, Lima, Peru; MCL11238	10	5	1	4	8	8	7	13	S		R	—
36	Post-operative, back, Tacoma, WA, USA; MCL8040	1	2	5	2	1	8	3	14	S		R	+
37	Blood, Mexico City, Mexico; MCL11657	1	2	6	2	1	7	1	15	1		R	+
38	Exudate right inguina, Sun City, AZ, USA; MCL8778	1	8	2	2	1	1	1	16	1		S	+
39	Vaginal swab, Kalafong Hospital, South Africa; MCL13231	1	9	4	3	6	9	2	17	S		S	—
40	Left leg, Springfield, IL, USA; MCL8834	4	5	1	2	4	4	5	18	S		R	—
41	Peritoneal fluid, Somers Pt, NJ, USA; MCL8566	5	4	1	2	1	8	4	19	S		R	—
42	Skin left leg, Ft Myers, FL, USA; MCL8544	6	6	7	5	4	5	5	20	S		S	—
43	Right leg wound, Baltimore, MD, USA; MCL8798	7	7	2	6	1	6	6	21	S		R	—
44	Appendix pus, Brazil; MCL10233	8	6	7	1	9	11	5	22	S		R	—
45	Endometrium, Los Angeles, CA, USA; MCL10147	1	2	2	2	1	1	3	23	1		S	+
<i>Staphylococcus xylosus</i> ATCC 35033	ATCC strain	—	—	—	—	—	—	—	—	—	—	—	

<i>Staphylococcus saprophyticus</i> MB4742	NRRL B2628	–	–	–	–	–	–	–	–	–	–
<i>Staphylococcus capitis</i> :											
MB5230	ATCC 27840 ^T	–	–	–	–	–	–	–	–	–	–
MB5347	ATCC 27843	–	–	–	–	–	–	–	–	–	–
<i>Staphylococcus hominis</i> MB5231	ATCC 27844 ^T	–	–	–	–	–	–	–	–	–	–
<i>Staphylococcus sciuri</i> MB5349	ATCC 29060	–	–	–	–	–	–	–	–	–	–
<i>Staphylococcus haemolyticus</i> MB5411	Wound, Grand Forks, ND, USA	–	–	–	–	–	–	–	–	–	–
<i>Staphylococcus aureus</i> COL	MRSA strain	–	–	–	–	–	–	–	–	–	–

Table 2. Genes and PCR primers used for *S. epidermidis* MLST

Gene	GenBank no.	PCR primers	Amplified fragment size (bp)	Allele region (bp)	No. alleles
Shikimate dehydrogenase (<i>aroE</i>)	AF269525	Forward, TCTCATTTCATTATCGCCATTG; reverse, CTAAAGTATGTGAAGCTAAACG	586	482	10
Glycerol kinase (<i>glpF</i>)	AF270012	Forward, GGAGGAATGTATATGAATGC; reverse, GAATTGGCAAAATAGCATGTG	616	450	9
Guanylate kinase (<i>gmk</i>)	AF270133	Forward, CTGTTAATTGTTCTTTCAGGCC; reverse, ATTTAGCTTCGATTTCGCTCTC	574	469	7
Phosphate acetyltransferase (<i>pta</i>)	AF269661	Forward, TTAAGGAGGATATTATGGCTG; reverse, GCTTAACATAGCTACTTTAGG	620	499	6
Triphosphate isomerase (<i>tpi</i>)	AF269838	Forward, CAATCCAATTAGACGCTTTAG; reverse, TAATGATGCGCCACCTACAAG	593	424	9
Acetyl coenzyme A acetyltransferase (<i>yqil</i>)	AF270323	Forward, CACGCATAGTATTAGCTGAAG; reverse, GTTGTGGTACCTCTACAGGTAC	615	473	11
Heat-shock protein 60 (<i>hsp60</i>)	AF029245	Forward, GTTTTAGCACAATCAATGATTTCAG; reverse, GCATCGCCTTCTACTTCATCC	491	421	7

RESULTS AND DISCUSSION

BLAST searches were done in GenBank with the seven MLST alleles used for typing *S. aureus*, as described by Enright *et al.* (2000). Sequence equivalents were found in *S. epidermidis* for all of the alleles except for the carbamate kinase (*arcC*) allele. Heat-shock protein (Hsp) was included in the study to ensure that the analysis was done with the appropriate number of alleles (seven). PCR primers (Table 2) were successful in amplifying PCR products of the expected length from all *S. epidermidis* strains tested. The same PCR primers were used to screen a strain of *S. aureus* and seven non-*S. epidermidis* CoNS strains, to test the specificity of the PCR primers for *S. epidermidis* strains. No PCR products were obtained for the *S. aureus* strain or for the majority of CoNS strains (Table 3). Where PCR products were obtained, they were not of the expected size. Sequence analysis of these products revealed that they were not the target sequences.

PCR products obtained from the *S. epidermidis* allelic screen were all sequenced (both strands) and analysed for allelic variation. The number of different variants for each locus ranged from six to ten. The most variable locus was *aroE*, which had a total of 28 polymorphic sites; *tpi* was the least variable with 12 polymorphic sites. The allelic profile obtained for each *S. epidermidis* strain is given in Table 1. In total, 23 STs were identified for the 47 isolates (Table 1). The largest ST group (group 2) contained 17 isolates; there were five additional groups that contained more than one strain and the remaining 17 ST groups contained single isolates. STs were compared by cluster and BURST analyses. For BURST analysis, a clonal group was considered to be strains with an ST that shared at least six alleles. One clonal group that contained 11 STs (representing 34 isolates) was detected. All other STs were non-clonal ('singletons'). BURST groupings were confirmed by cluster analysis, which showed

that isolates within this group displayed 76 % similarity (Fig. 1). The most divergent STs were 5, 17, 20 and 22, which all fell away from the main cluster with no shared alleles.

PFGE with *SmaI*-digested DNA was performed on the isolates to determine how the two methods compared. The largest ST group (group 2) contained strains with *SmaI* restriction profiles that fell into four groups (Fig. 2a). The remaining ST groups that contained more than one isolate had similar profiles within their ST groups, with differences that ranged from none to two bands (Fig. 2b). Cluster analysis of all *SmaI* restriction profiles demonstrated the similarity of the isolates within the ST groups to which they were assigned, with just two groups (2 and 3) clustering away from the corresponding group members (Fig. 2c). Two main clusters were resolved, one of which contained six 'singleton' BURST clonal groups and one that contained a mixture of 'singleton' BURST clonal groups and BURST clonal group 1 isolates. The clustering pattern was similar to that obtained for the MLST profiles (Fig. 1).

Methicillin-resistance data were provided for all isolates in the collection; 31 (67 %) were reported to be resistant (Table 1). To confirm these results, isolates were also probed for the presence of the *mecA* gene by Southern hybridization; by using this method, 33 (70 %) contained the gene. Inconsistencies with MIC values were attributed to these being determined in several different laboratories. Strains were deemed to be resistant if they contained the *mecA* gene, in accordance with NCCLS guidelines. The resistance status of each strain is also marked on the MLST and PFGE dendrograms (Figs 1 and 2c). The methicillin-resistance genotype appeared to be randomly distributed among strains, suggesting that it has been acquired randomly. The largest ST group (group 2) contained 14 *mecA*-positive strains and three that were negative; the gene was not confined to specific PFGE profiles within this group. It was also observed that isolates

Table 3. Specificity of MLST primers for *S. epidermidis*

s, PCR product size is similar to that of *S. epidermidis* by gel electrophoresis; however, sequence is not *S. epidermidis*-like (21–27 % mismatch in *pta* and 15–18 % mismatch in *hsp*); N, PCR product sizes were different from that of *S. epidermidis*; –, no amplification was observed.

Species	Locus						
	<i>aroE</i>	<i>glpF</i>	<i>gmk</i>	<i>pta</i>	<i>tpi</i>	<i>yqiL</i>	<i>hsp</i>
<i>S. epidermidis</i>	586*	616	574	620	593	615	491
<i>S. xylosus</i>	N	—	N	N	N	N	—
<i>S. saprophyticus</i>	N	—	N	S	N	—	S
<i>S. capitis</i>	N	—	N	S	N	—	S
<i>S. hominis</i>	N	—	N	N	N	—	S
<i>S. sciuri</i>	N	—	N	N	N	N	N
<i>S. haemolyticus</i>	N	—	N	N	N	—	N
<i>S. aureus</i> COL	N	—	—	N	N	—	S

*Size of sequence (bp).

Fig. 1. Dendrogram showing genetic relatedness of 47 *S. epidermidis* isolates. Data were compared by using the simple matching coefficient and clustered by UPGMA.

from the singleton group were predominantly *mecA*-negative; however, as they were not represented by multiple isolates, this observation may not be significant.

S. epidermidis isolates used in this study originated from a diverse range of infections and geographical locations and were cultured in the years 1996–1999. Thirty-three isolates were obtained from continental USA, one from Hawaii, one from Europe, five from South America and seven from South Africa. In total, 23 different STs were found, five of which

contained more than one isolate. No correlation was observed between an isolate's ST and its infection site or geographical location. The largest group (group 2) was composed of strains from continental USA, Europe and South Africa. STs 3 and 4 contained isolates from South Africa and continental USA; ST 5 contained isolates from Brazil and continental USA and isolates from ST 8 were from Hawaii and continental USA. Within BURST clonal group 1, there was also a global distribution of strain types, with isolates from the USA, Europe, South Africa and Mexico.

Fig. 2. PFGE analysis of isolates by using *Sma*I restriction digests. (a) Subgroupings within ST 2: lanes 1 and 19, λ ladder; lane 2, *S. epidermidis* 1; lane 3, *S. epidermidis* 22; lane 4, *S. epidermidis* 3; lane 5, *S. epidermidis* 4; lane 6, *S. epidermidis* 6; lane 7, *S. epidermidis* 2; lane 8, *S. epidermidis* 7; lane 9, *S. epidermidis* 9; lane 10, *S. epidermidis* MB2230; lane 11, *S. epidermidis* 45; lane 12, *S. epidermidis* 8; lane 13, *S. epidermidis* 10; lane 14, *S. epidermidis* 11; lane 15, *S. epidermidis* 14; lane 16, *S. epidermidis* 12; lane 17, *S. epidermidis* 15; lane 18, *S. epidermidis* 13. (b) All PFGE profiles obtained, according to ST group: lanes 20, 37, 38 and 54, λ ladder; lane 21, RP62A; lane 22, *S. epidermidis* 10; lane 23, *S. epidermidis* 12; lane 24, *S. epidermidis* 3; lane 25, *S. epidermidis* 7; lane 26, *S. epidermidis* 19; lane 27, *S. epidermidis* 20; lane 28, *S. epidermidis* 17; lane 29, *S. epidermidis* 18; lane 30, *S. epidermidis* 21; lane 31, *S. epidermidis* 22; lane 32, *S. epidermidis* 23; lane 33, *S. epidermidis* 24; lane 34, *S. epidermidis* 25; lane 35, *S. epidermidis* 28; lane 36, *S. epidermidis* 27; lane 39, RP62A; lane 40, *S. epidermidis* 29; lane 41, *S. epidermidis* 31; lane 42, *S. epidermidis* 32; lane 43, *S. epidermidis* 33; lane 44, *S. epidermidis* 34; lane 45, *S. epidermidis* 35; lane 46, *S. epidermidis* 36; lane 47, *S. epidermidis* 37; lane 48, *S. epidermidis* 38; lane 49, *S. epidermidis* 39; lane 50, *S. epidermidis* 40; lane 51, *S. epidermidis* 42; lane 52, *S. epidermidis* 43; lane 53, *S. epidermidis* 44. ST groups that contain multiple isolates are marked. ST2 is represented by one strain from each of the PFGE subgroups. (c) Cluster analysis of *Sma*I restriction profiles for *S. epidermidis* isolates. Analysis was performed by using the Jaquard coefficient and clustered by UPGMA.

Isolates that did not correspond to a clonal group were derived from continental USA, Brazil, Peru and South Africa. As this was a pilot study (47 isolates, of which 33 were from continental USA), one cannot make generalized conclusions about the epidemiology of *S. epidermidis* disease. However, it does demonstrate that clonal types are distributed globally and that there does not appear to be a correlation between ST and diagnosed infection caused by the isolate. The clonal dissimilation of *S. epidermidis* strains is supported by the work of Miragaia *et al.* (2002), who used PFGE to type a large collection of isolates from Denmark ($n = 136$) and Iceland ($n = 94$) and detected 40 different clonal groups. Comparison of these groups with strains from Mexico ($n = 74$), Uruguay ($n = 10$), Greece ($n = 34$) and Cape Verde ($n = 48$) revealed that isolates had similar PFGE profiles that were independent of their geographical location.

To compare MLST with an established typing method, we also analysed the *S. epidermidis* isolates by PFGE. Twenty-three patterns were observed by using the criteria proposed by Tenover *et al.* (1995). Taken alone, the PFGE data suggest a high degree of genotypic diversity within the *S. epidermidis* isolates studied. This mirrors other studies that have sought to type *S. epidermidis* strains by PFGE. Raimundo *et al.* (2002) typed 55 isolates from a neonatal intensive-care unit (NICU) and identified 43 subtypes by PFGE, compared to only 15 types by RAPD analysis. Sloos *et al.* (1998) typed 53 isolates from 24 neonates and found that 16 isolates (from six babies) had common PFGE profiles, whereas the remaining 37 isolates all gave unique profiles. In a second study, Sloos *et al.* (2000) characterized 16 epidemiologically distinct isolates by PFGE, all with unique profiles. We have demonstrated that MLST provides a more defined picture of taxonomic diversity, with 74.5 % of strains originating from a single BURST clonal group and the remaining 13 types all being 'singletons'. The genetic diversity observed from the PFGE profiles suggests that *S. epidermidis* strains undergo a high degree of gene flux, more so than other species that have been typed by using MLST, as multiple PFGE types were not observed within single ST groups (Enright *et al.*, 2000). It is to be expected that greater variation is observed by using PFGE than MLST, as MLST is a measure of genetic change for housekeeping genes, which evolve extremely slowly due to reduced tolerance for change. PFGE measures more rapid changes and the rate of gene exchange between micro-organisms is only now being fully appreciated, with the advent of whole-genome sequencing (Cerdeño-Tárraga *et al.*, 2001).

S. epidermidis strains are often considered to be part of the normal flora of human skin and as such are distributed ubiquitously (von Eiff *et al.*, 2002). In this study, isolates either belonged to a single clonal group or were singletons. As the *S. epidermidis* database increases, it will be extremely interesting to see whether clonal groups also develop for the singleton isolates or there is a predominance of a single group, and whether that group is more invasive than others.

S. epidermidis epidemiology studies have been limited

historically, due to the fact that *S. epidermidis* strains are often considered to be contaminants, as opposed to the disease-causing organism (Herwaldt *et al.*, 1996), and they are usually grouped with other CoNS species. However, *S. epidermidis* infections are a rising cause of concern, due to the high distribution of methicillin resistance [some studies report up to 73 % (Petinaki *et al.*, 2001)] and their persistence on indwelling devices, often resulting in replacement of the device, which causes more trauma and is costly (Hakim *et al.*, 2000). In this investigation, 70 % of isolates were methicillin-resistant. *S. epidermidis* is also the leading cause of nosocomial infections in neonates. Villari *et al.* (2000) reported an infection rate of 18.7 % between 1996 and 1998 for a NICU; of these infections, 30 % were due to *S. epidermidis*, including bloodstream infections (39.8 % of bloodstream infections), surface infections such as conjunctivitis (29.8 %) and meningitis (58.3 % of cases).

S. epidermidis has also not received as much attention as other pathogenic micro-organisms by the various pathogen sequencing projects. The sequence of *S. epidermidis* ATCC 12228 is now complete (GenBank accession no. AE015929) and *S. epidermidis* RP62A is being sequenced by the Institute of Genomic Research (www.tigr.org). Our MLST analysis demonstrated that this strain is a singleton and is related only distantly to the majority of disease-causing strains, which were more similar to *S. epidermidis* ATCC 12228.

In this investigation, we demonstrated that a novel MLST scheme could be used to differentiate *S. epidermidis* isolates and act as a tool for discrimination of *S. epidermidis* strains from other CoNS species. As such, it will be useful for identifying divergent strains and thus will have some utility for demonstrating the target range for anti-*S. epidermidis* agents, such as vaccines. Work is ongoing to increase the *S. epidermidis* MLST database. MLST will not, however, be useful as a stand-alone CoNS typing method in clinical settings; it is not as discriminatory as PFGE and it is too costly and time-consuming to replace PFGE. Its utility is that it can be incorporated into polyphasic approaches for *S. epidermidis* epidemiology and pathogenicity studies. Peacock *et al.* (2002) used MLST in combination with specific pathogenicity genes to identify virulent strains of *S. aureus*. It will now be possible to do similar studies with *S. epidermidis*.

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