

ORIGINAL ARTICLE

A diversity profile from the staphylococcal community on atopic dermatitis skin: a molecular approach

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atopic dermatitis, genotyping, polymerase chain reaction, Staphylococci, superantigen.

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Abstract**Aims:** The aim of this study was to determine the biodiversity of the skin staphylococcal community from patients with atopic dermatitis (AD) and superantigen (SAG) detection from *Staphylococcus aureus* isolates.**Methods and Results:** In this study, we developed a novel multiplex PCR that allows the identification and discrimination of bacteria belonging to the *Staphylococcus* genus both *Staph. aureus* and coagulase-negative *Staphylococcus* – *Staph. capitis*, *Staph. epidermidis*, *Staph. haemolyticus* and *Staph. hominis* isolated from the skin of patients with AD. In addition, a multiplex PCR assay that allows the rapid screening of the 19 genes that encode *staphylococcal* enterotoxins (SEs), SE-like toxins and toxic shock syndrome toxin-1 was also performed and applied in *Staph. aureus* isolates. The microflora of the skin of patients with AD was dominated by *Staph. aureus* (69 isolates, 35.6%) followed by *Staph. epidermidis* (59 isolates, 30.4%) species. The SEIM and SEIN genes were the most frequently detected in our study (15 isolates, 71.4%), followed by SEG and SEIO (14 isolates, 66.7%).**Conclusions:** Our molecular-based approach successfully identified the staphylococcal microflora that was relatively specific to patients with AD. Considering skin colonization and expression of virulence factors, the *Staph. aureus* may play a relevant role in AD pathophysiology.**Significance and Impact of the Study:** This ability to classify disease-related microbial species provides new insights into the relevance of those microbes in human disorders.**Introduction**

Skin is a complex and dynamic ecosystem inhabited by a large multitude of micro-organisms. The composition of the human skin microbiota is influenced by host demographics and genetics, human behaviour, local and regional environmental characteristics and transmission events. This variance could definitively influence human health and disease outcomes among individuals. Therefore, the biodiversity profile of the human microbiota may be predictive or diagnostic of some disease states (Rosenthal *et al.* 2011).

The human-skin-resident microflora is mainly composed of the *Staphylococcus*, *Corynebacterium*, *Propionibacterium*, *Micrococcus*, *Brevibacterium*, *Acinetobacter* Genera (Cogen *et al.* 2008).

Atopic dermatitis (AD) is an inflammatory skin disorder, chronically relapsing and genetically linked. The occurrence of AD is 10–20% and 1–3% in children and adults worldwide, respectively (Leung and Bieber 2003). It is the most frequent chronic skin disorder in children, with an increasing incidence and a considerable, unfavourable impact on the quality of life. This is a multifactorial skin disorder, which consists of complex connections among susceptibility genes, ecological factors and allergens, skin barrier dysfunction, and uncharacteristic systemic and local immune reactions (Lin *et al.* 2007; Lopes *et al.* 2011). Mutations in the gene that produces the skin barrier protein filaggrin are associated with AD, predominantly in patients, which consequently develop asthma and/or allergic rhinitis, suggesting that epicutaneous

sensitization may provide atopic disease (Palmer *et al.* 2006; Sandilands *et al.* 2007). These patients may experience repeated skin infections with increased numbers of pathogenic bacteria, such as *Staphylococcus aureus*; however, nonpathogenic bacteria usually present on the skin surface could take part in a protective role. The diminished expression of antimicrobial peptides on patients with AD skin might contribute to this vulnerability (Ong *et al.* 2002). The potential pathogenic *Staph. aureus* is not recognized as a member of the resident skin microflora. In healthy individuals, the frequency of *Staph. aureus* skin colonization is about 5%, whereas the frequency of *Staph. aureus* skin colonization is more than 90% in patients with AD in both the lesional and nonlesional skin: quantitatively, *Staph. aureus* counts could differ by 100- to 1000-fold between lesional skin and nonlesional skin (Lin *et al.* 2007). Moreover, the colonization rate and density by *Staph. aureus* species on lesional skin are also considerably correlated with the clinical severity of AD disease (Leyden *et al.* 1974; Hauser *et al.* 1985; Lin *et al.* 2007). In addition, more than 70% of *Staph. aureus* strains from AD skin are exotoxin producers and are able to secrete various exotoxins with superantigen (SAg) activity (Hoeger *et al.* 1992; Akiyama *et al.* 1996; Lin *et al.* 2007). These exotoxins might go through the skin barrier and contribute to the perseverance and exacerbation of the allergic state (Bunikowski *et al.* 2000). Various studies also indicate a positive correlation between the clinical severity and the colonization by SAg-producing strains of *Staph. aureus* in AD (Lin *et al.* 2007; Zuel-Fakkar and El-Shokry 2010; Nada *et al.* 2012). Thus, a more thorough understanding of skin staphylococcal community in patients with AD may provide a foundation regarding the clinical management of staphylococcal infections in AD. Additionally, to our knowledge, no study has been performed on the skin of the staphylococcal community of Portuguese patients with AD.

To study the biodiversity of the skin staphylococcal community in AD, we used a molecular-based approach, which enabled rapid analysis of the staphylococcal composition from the skin of Portuguese patients with AD. Simultaneously, a multiplex PCR system detecting 19 types of SAg genes, such as staphylococcal enterotoxins (SEs), SE-like toxins (SEls) and toxic shock syndrome toxin-1 (TSST-1), was applied to the *Staph. aureus* isolates.

Materials and methods

Study design

The study population comprised nine patients attending an allergy clinic with medical diagnosis of AD according to Hanifin and Rajka (1980) criteria (aged 3–35 years),

Table 1 Baseline characteristics of participants

Baseline characteristics	Patients with AD	Healthy controls
Total subjects analysed	9	24
Total samples analysed*	18	24
Age, mean (range) (years)	15 (3–35)	22 (19–33)
Male/female	4/9	11/24
Rhinitis	9/9	0
Asthma	6/9	0
Food allergy†‡	2/9	0
Dust mite allergy†	8/9	0
Pollen allergy†	6/9	0

*Within patients with AD, the samples were obtained from the antecubital and popliteal crease, and in healthy individuals, the samples were obtained only from antecubital crease.

†Confirmed by skin prick tests and/or specific IgE measurements.

‡Peanuts and nuts in one patient and milk and egg allergy in one patient.

with clinically apparent AD lesions without signs of secondary infection, and consecutively included. Patients were not taking systemic antibiotics, topical corticosteroids, calcineurin inhibitors or immunosuppressants during the previous 2 weeks of medical observation and sampling. All patients had moderate-to-severe disease, and their atopy status was characterized. Their baseline characteristics are described in Table 1.

Samples from the antecubital crease of 24 healthy controls obtained through a previously reported research (Tavaria *et al.* 2012) were also integrated in this study.

Sample collection

The sampling procedure was performed in 25 cm² of the skin area on the sampling sites (popliteal and/or antecubital crease). The skin within the enclosed area was scrubbed using a sterile swab moistened with dilution liquid. The tip of the swab was then broken against the wall of a glass tube containing the dilution liquid, and the tube was immediately capped and shaken to suspend the bacteria. The samples were cultured in Baird Parker medium (BPM; Lab M, Lancashire, UK) by spread plate technique in duplicate and incubated at 37°C during 24–48 h. The micro-organism isolation procedure was performed as described by Soares *et al.* (2011). A total of 194 isolates were obtained from the skin of patients with AD and 48 isolates from the skin of healthy individuals, isolated and selected according to Tavaria *et al.* (2012).

DNA preparation

DNA was isolated as described by Soares *et al.* (2011).

Reference strains

The type strains used in this study were as follows: *Staph. aureus* ATCC 25923, *Staph. epidermidis* ATCC 14990, *Staph. capitis* subsp. *capitis* ATCC 27840, *Staph. haemolyticus* ATCC 29970 and *Staph. hominis* subsp. *hominis* ATCC 27844. In addition, the type strains of *Staph. aureus* that are described in Soares *et al.* (2011) were used as positive controls for SAg genes.

Molecular identification of the staphylococcal isolates

Multiplex PCR

The wild isolates obtained were submitted to multiplex PCR with the primers FemF/FemR (Mehrotra *et al.* 2000), SepF/SepR (Martineau *et al.* 1996), ScapF/ScapR (Iwase *et al.* 2007), ShaemF/ShaemR (Schuenck *et al.* 2008) and ShomF/ShomR (Vannuffel *et al.* 1999) for the identification of the *Staph. aureus*, *Staph. epidermidis*, *Staph. capitis*, *Staph. haemolyticus* and *Staph. hominis* species, respectively. The list of the primers used and the PCR products generated is described in Table 2. Multiplex PCR and amplification were performed as described by Tavaría *et al.* (2012).

sodA amplification and sequencing

Complementary identification of three (or one in the case of *Staph. lentus*) isolates for each species formerly identified by multiplex PCR was performed by *sodA* gene sequencing as described by Tavaría *et al.* (2012).

Multiplex PCR for the detection of SAg genes

Twenty one of the 69 isolates previously identified as *Staph. aureus* strains from Portuguese patients with AD were screened for SAg gene detection. They were randomly selected and consist of five to six isolates per individual positive for *Staph. aureus*. The primers were

first applied individually, and multiplex PCR conditions were prepared as described by Hwang *et al.* (2007).

Other virulence factors

For virulence factor detection, namely coagulase production, DNase and haemolytic activity, strains were tested as demonstrated by Soares *et al.* (2011).

Results

Staphylococcus identification

From the AD subjects analysed ($n = 9$), we obtained six individuals with skin samples positive for *Staphylococcus* spp. as determined by the BPM counts. After preliminary screening for Gram-positive and catalase-positive and to distinguish among staphylococcal species, we generated a multiplex PCR to uniquely classify the isolates.

To ensure a good amplification with the five primer pairs in the same PCR run, the reaction conditions for the multiplex PCR assays were optimized, which resulted in a strong and reproducible amplification with primers used. The specificity of the multiplex PCR was assessed with a panel of five reference strains, and the results of the multiplex PCR amplification are reported in Fig. 1. The five reference strains of the *Staphylococcus* genus were identified as *Staph. aureus*, *Staph. epidermidis*, *Staph. capitis*, *Staph. haemolyticus* and *Staph. hominis* by yielding specific DNA fragments of sizes 723, 124, 208, 271 and 866 bp, respectively. From these 194 isolates, 69 (35.6%) were identified as *Staph. aureus* by yielding a PCR product of 723 bp. The proportion of *Staph. epidermidis* was also significantly higher with 59 (30.4%) isolates identified; 54 (27.8%) isolates were identified as *Staph. hominis*, which gave an amplification product of 866 bp; 12 (6.2%) isolates were recognized as *Staph. capitis* by yielding a PCR product of 208 bp. Thus, four different species

Table 2 DNA oligonucleotide sequences used as primers and expected size of PCR products used in the multiplex PCR for *Staphylococcus* identification

Primers	Oligonucleotide sequence 5'-3'*	Target species	Amplicon bp	Reference
FemF	ACAGCTAAAGAGTTTGGTGCCTxxxxGATAGCATGC	<i>Staphylococcus aureus</i>	723	Mehrotra <i>et al.</i> (2000)
FemR	TTCATCAAAGTTGATATACGCTAAAGGTxxxxCACACGGTC			
SepF	ATCAAAAAGTTGGCGAACCTTTTCA	<i>Staphylococcus epidermidis</i>	124	Martineau <i>et al.</i> (1996)
SepR	CAAAGAGCGTGGAGAAAAGTATCA			
ScapF	GCTAATTTAGATAGCGTACCTTCA	<i>Staphylococcus capitis</i>	208	Iwase <i>et al.</i> (2007)
ScapR	CAGATCCAAAGCGTGCA			
ShaemF	GGTCGCTTAGTCGGAACAAT	<i>Staphylococcus haemolyticus</i>	271	Schuenck <i>et al.</i> (2008)
ShaemR	CACGAGCAATCTCATCACCT			
ShomF	TGCCATATAGTCATTTACG	<i>Staphylococcus hominis</i>	866	Vannuffel <i>et al.</i> (1999)
ShomR	GTTCTAATTGAAGTTGTGTTG			

*Underlined base pairs are polydeoxyinosine bridge region.

were recognized because the *Staph. haemolyticus* species was not identified in this study. The cultivation approach has confirmed a higher possibility of *Staph. aureus* colonization in AD skin given that the proportion of *Staph. aureus* detection (4 of 6 patients with AD) was significant as described in Fig. 2.

To assess how distinct the staphylococcal skin flora for AD might be, we compared it with isolates from 24 healthy individuals (Tavaria et al. 2012). The samples from healthy individuals were characterized by a greater heterogeneity in terms of the number of identified species, viz. *Staph. aureus* (eight isolates), *Staph. capitis* (four isolates), *Staph. epidermidis* (four isolates), *Staph. haemolyticus* (five isolates) and *Staph. hominis*

(four isolates), *Staph. lentus* (one isolate), *Staph. lugdunensis* (three isolates), *Staph. saprophyticus* (nine isolates) and *Staph. warneri* (10 isolates) as seen in Fig. 2.

SAg genes detection

The primer sets successfully amplified the target genes in the multiplex PCR without nonspecific or additional bands on the reference strains (data not shown). All of the PCR products showed the expected size when tested individually and were coordinated with the results described by Løvseth et al. (2004) and Hwang et al. (2007). In this study, 21 *Staph. aureus* strains isolated from AD skin were tested, and sixteen (76%) of them were SAg-positive strains (Fig. 3). The most frequently detected genes were SEG, SEIM, SEIN and SEIO (15 isolates, 71%), and they were always found together in the same isolate, with the exception for one isolate where we did not detect the SEIO gene.

SEA (six isolates, 29%) and SEIL (seven isolates, 33%) were also frequently detected along with the SEG, SEIM, SEIN and SEIO genes. The classical SEs (SEA-SEE) were not detected in our samples. The SEIU gene (one isolate, 5%), which was rarely detected, was also found together with the SEG, SEIM, SEIN and SEIO genes. Based on the SAg genotype of *Staph. aureus* isolates, we obtained six different genotypes with SEG, SEIM, SEIN with SEIO combination being the most predominant (eight isolates) followed by SEA, SEG, SEIM, SEIN, SEIL with SEIO combination with three isolates.

Other virulence factors

Sixty-nine (35.6%) and eight (16.7%) isolates among AD and control individuals, respectively, were positive for

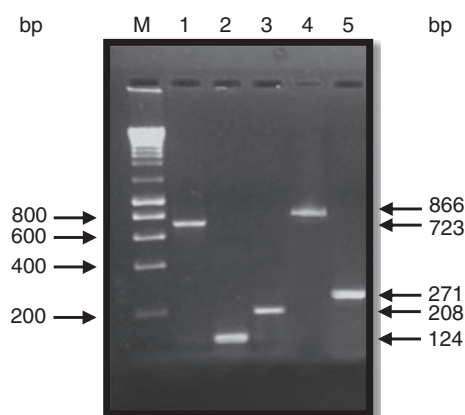


Figure 1 Multiplex PCR amplifications obtained with *Staphylococcus aureus* -, *Staph. epidermidis*-, *Staph. capitis*-, *Staph. haemolyticus*- and *Staph. hominis*-specific primers. Lane 1, *Staph. aureus* ATCC 25923; lane 2, *Staph. epidermidis* ATCC 14990; lane 3, *Staph. capitis* subsp. *capitis* ATCC 27840/ATCC 15305; lane 4, *Staph. haemolyticus* ATCC 29970; lane 5, *Staph. hominis* subsp. *hominis* ATCC 27844; and lane M, 200-bp marker NZYTech.

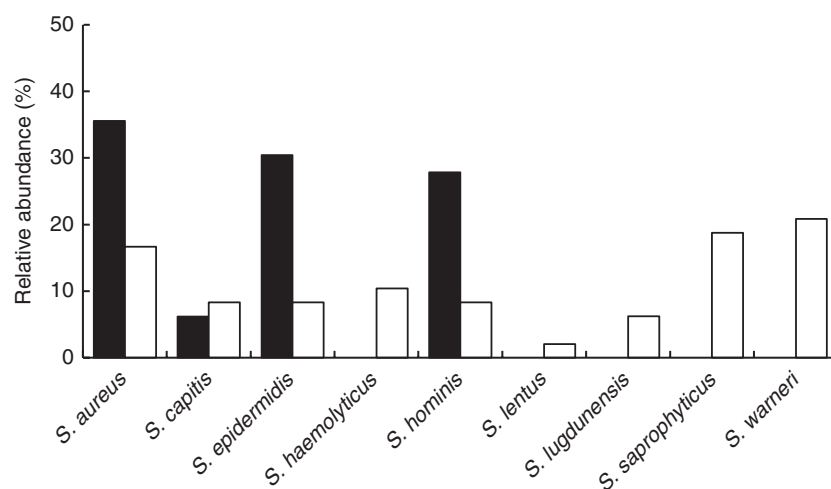


Figure 2 Bacterial taxonomic classifications with mean relative abundance of the staphylococcal species identified from the skin of patients with AD. Non-AD controls are also included, consisting of two isolates per individual from 24 healthy individuals as previously demonstrated by Tavaria et al. (2012). (■) AD patients and (□) non-AD controls.

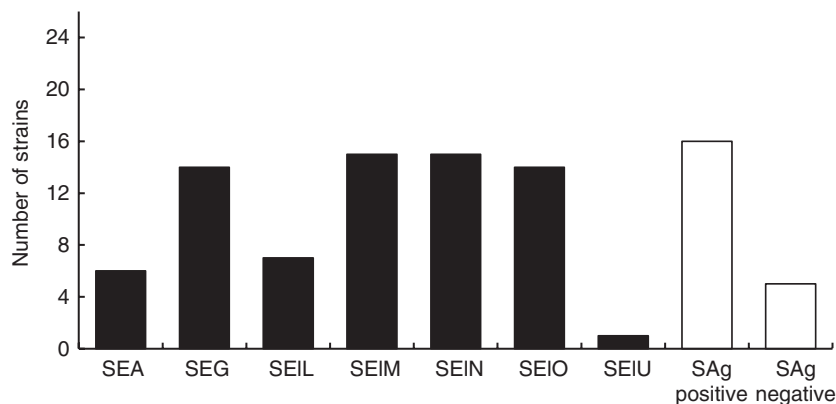


Figure 3 Diversity of SAg genes detected and prevalence of toxigenic and nontoxigenic strains of *Staphylococcus aureus* isolated from the skin of Portuguese patients with AD.

both the presence of coagulase and DNase and correlated with the *Staph. aureus* isolates as expected. In terms of haemolytic activity as performed in horse blood agar, a similar occurrence was seen in control (39.6%) and AD individuals (33.7%).

Discussion

Atopic dermatitis is a frequent inflammatory skin disorder generally associated with the antecubital and popliteal regions, sites that possess identical groups of micro-organisms, but different proportions of such microbial communities (Grice *et al.* 2009). These findings suggest the preferential role of some micro-organisms in skin disorders and their predisposition for conventional sites in human body (Kong *et al.* 2012). AD is typically treated with antimicrobial approaches, and therefore, this is an example of a disorder in which the involvement of micro-organisms in the course of the disease can be assessed by microbial community analyses (Gloor *et al.* 1982; Kong *et al.* 2012).

To classify the staphylococcal population isolated from the skin of patients with AD, multiplex PCR targeting predominant species could be the method of choice for rapid and reliable identification. To our knowledge, no previous study exploited this molecular approach for the identification of staphylococcal strains isolated from the skin.

Using direct multiplex PCR, we defined and characterized the staphylococcal skin community in six patients with AD. To distinguish the five species, which could have a relevant interest for AD, the multiplex PCR-based approach associated five pairs of primers amplifying species-specific fragments designed for *Staph. aureus* (Mehrotra *et al.* 2000), *Staph. epidermidis* (Martineau *et al.* 1996), *Staph. capitis* (Iwase *et al.* 2007), *Staph. haemolyticus* (Schuenck *et al.* 2008) and *Staph. hominis* (Vannuffel *et al.* 1999). These primers were selected based on their thermodynamical compatibility and on the production of PCR bands distinct in size.

From the six individuals positive for *Staphylococcus* spp., as determined by the BPM counts, the staphylococcal microflora was dominated by *Staph. aureus* (69 isolates, 35.6%) followed by *Staph. epidermidis* (59 isolates, 30.4%) and *Staph. hominis* (54 isolates, 27.8%) species in patients with AD (Fig. 2). Although the diminutive study cohort, four patients (67%) were found to be colonized with *Staph. aureus* in their skin. These results were in agreement with those reported by Lin *et al.* (2007), which demonstrated that 75–100% of patients with AD have *Staph. aureus* on their lesional skin. Moreover, a major feature of AD is the presence of *Staphylococcus* species in very high numbers (10–100 times larger than that of normal individuals) (Gloor *et al.* 1982).

The *Staphylococcus* species detected in this study includes both coagulase-negative staphylococci, a major member of normal microbiota, and *Staph. aureus*, a potential pathogen. The genus *Staphylococcus* has several bacterial species relevant to the clinical disease, which includes *Staph. epidermidis* as a skin commensal and *Staph. aureus* as a micro-organism associated with AD (Kong *et al.* 2012). However, in this study, we have extended these observations by demonstrating simultaneous fluctuations in other skin bacteria. *Staph. epidermidis* is considered as the principal *Staphylococcus* species in the skin of controls (Iwase *et al.* 2010; Lai *et al.* 2010). The simultaneous prevalence of both *Staph. aureus* and *Staph. epidermidis* in Portuguese patients with AD was also observed in this study, which could provide a novel approach into the association between staphylococci as reported recently by Kong *et al.* (2012). These two species could possibly share a mutualistic or commensal association to improve frequent resistance to antimicrobial peptides (Peschel *et al.* 2001; Sieprawska-Lupa *et al.* 2004; Lai *et al.* 2007; Li *et al.* 2007) or improve binding to exposed extracellular matrix proteins in inflamed AD skin (Nilsson *et al.* 1998; McCrea *et al.* 2000; Cho *et al.* 2001; Williams *et al.* 2002). Otherwise, it might represent a compensatory mechanism of increasing *Staph. epidermidis*

numbers in an effort to control *Staph. aureus* (Iwase et al. 2010; Kong et al. 2012). To our knowledge, *Staph. hominis* species has never been reported as an important micro-organism in AD, but in our study, they constitute a relevant fraction of the isolates (27.8%), which possibly could reflect new approaches into the association between staphylococci species similar to those described above for the *Staph. epidermidis* species.

In a previously work (Tavaria et al. 2012), we characterized the staphylococcal community of 24 healthy Portuguese individuals by the same molecular-based approach resulting in 48 isolates included here as controls. Comparing these results with those obtained with patients with AD, a shift in the predominant strains and in the diversity of the skin microflora was seen, with nine species being detected in healthy individuals in contrast to only four species identified in AD. Therefore, the staphylococci identified in the present study may suggest a better adaptation of these strains to the AD environment. However, a cautious note should be made due to the lower number of isolates per individual, increasing interpersonal heterogeneity, which could in part influence the higher biodiversity of normal skin. However, Kong et al. (2012) also demonstrated in their study of the skin microbiome in children with AD, where increase in *Staph. aureus* accounted for reductions in skin microbial biodiversity. In fact, as a result of a skin barrier dysfunction and inflammation of the upper dermis, the skin of patients with AD exhibits a remarkable susceptibility to colonization with *Staph. aureus*.

The important role of colonization by *Staph. aureus* as an aggravating factor in AD is well established, as there is a significant correlation between the severity of the disease and *Staph. aureus* skin colonization (Bunikowski et al. 2000). Several strains of *Staph. aureus* present on atopic skin include a peculiar ability to produce exotoxins with SAg activity, which may play an important role in the natural course of atopic dermatitis.

Furthermore, the skin of patients with AD colonized with *Staph. aureus* harbouring SAg genes shows a significant increased severity of the disease as compared to patients colonized by *Staph. aureus* with no SAg genes (Nishijina et al. 1997; Roll et al. 2004; Nada et al. 2012). A multiplex PCR assay that allows for the rapid screening of the 19 genes that encodes SEs, SEIs and TSST-1 in 21 *Staph. aureus* strains isolated from the skin of patients with AD is described in this study. As result, 16 of the 21 *Staph. aureus* strains (76%) showed the presence of toxin genes, while the remaining five strains (24%) did not harbour toxin genes. Previous studies have shown that more than 50–60% of *Staph. aureus* strains isolated from patients with AD are exotoxin-producing strains, which can secrete various exotoxins including SEA, SEB, SEC,

SED and TSST-1 (Bunikowski et al. 2000; Chen et al. 2005; Silva et al. 2006; Schlievert et al. 2008; Nada et al. 2012).

More than one toxin gene was detected in all strains up to seven toxin genes detected simultaneously in one strain. The SEIM and SEIN (15 isolates, 71.4%) genes were the most frequently detected in our study, followed by SEG and SEIO (14 isolates, 66.7%). All these genes were found together in the same strain with only one exception. Those genes are components of the enterotoxin gene cluster (egc), and the previously known egc type (SEG, SEI, SEIM and SEIN with SEIO or SEIU) (Hwang et al. 2007) was frequently distinguished in this study. Other studies also revealed a high prevalence of SAg genes associated with the egc locus in *Staph. aureus* isolates from patients with AD (Mempel et al. 2003; Bonness et al. 2008; Schlievert et al. 2008).

Although SEI and SEG are encoded on the same pathogenicity island, the egc cluster, frequently only SEG, was detected using the primer pairs described previously (Hwang et al. 2007). This effect may be caused by polymorphisms that were found in the egc cluster (Mempel et al. 2003). Moreover, the SEA (six isolates), SEIL (seven isolates) and SEIU (one isolate) genes were also detected, but in low frequencies. Nada et al. (2012) proposed that this diversity in toxin detection in atopics is associated with the severity of the disease and the site of the skin involved or sampled. This was also confirmed by Yagi et al. (2004) in a study where *Staph. aureus* strains were isolated from different skin areas in patients with AD, finding that 41% in the nonlesional area, 62% in the dry lesional area and 75% in the exudative lesional area were SAg-positive. Despite the proportion of toxin-producing strains and the frequency of certain toxins differing between studies (Breuer et al. 2000; Schlievert et al. 2008), generally, the SEB or SEC is the most predominant SAg gene detected in AD (Bunikowski et al. 2000; Zuel-Fakkar and El-Shokry 2010; Nada et al. 2012). In our samples, none of these two types of SAg genes were detected, which may be attributed to the limited number of isolates and/or individuals we studied. However, Arkwright et al. (2001) also demonstrated that the SEB was poorly detected (4%) in the skin of children with atopic dermatitis.

The relevance of evaluating the combination of virulence traits among staphylococci has been recently emphasized in both human and veterinary medicine (Zecconi et al. 2006). Therefore, a similar proportion of the isolates showed haemolytic activity in horse blood agar both in healthy and AD individuals.

In conclusion, this molecular-based approach successfully identified the staphylococcal microflora that was relatively specific to patients with AD. Therefore, identification and

fluctuation of certain micro-organisms and their association with certain disorders reveal the importance of these microbes in the course of human disease. The presence of SAg genes among coagulase-negative Staphylococcus and other SAGs should be further studied to obtain a more comprehensive profile in patients with AD. Extending our observations to a large number of patients, with a longitudinal study design, may be also of great help to fully understand the role of the skin staphylococci community in the exacerbations and pathogenesis of atopic dermatitis.

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Conflict of Interest

The authors declare that they have no conflict of interest

References

- Akiyama, H., Toi, Y., Kanzaki, H., Tada, J. and Arata, J. (1996) Prevalence of producers of enterotoxins and toxic shock syndrome toxin-1 among *Staphylococcus aureus* strains isolated from atopic dermatitis lesions. *Arch Dermatol Res* **288**, 418–420.
- Arkwright, P.D., Cookson, B.D., Haeney, M.R., Sanyal, D., Potter, M.R. and David, T.J. (2001) Children with atopic dermatitis who carry toxin-positive *Staphylococcus aureus* strains have an expansion of blood CD52 B lymphocytes without an increase in disease severity. *Clin Exp Immunol* **125**, 184–189.
- Bonness, S., Szekat, C., Novak, N. and Bierbaum, G. (2008) Pulsed-Field gel electrophoresis of *Staphylococcus aureus* isolates from atopic patients revealing presence of similar strains in isolates from children and their parents. *J Clin Microbiol* **46**, 456–4561.
- Breuer, K., Wittmann, M., Boesche, B., Kapp, A. and Werfel, T. (2000) Severe atopic dermatitis is associated with sensitisation to staphylococcal enterotoxin B (SEB). *Allergy* **55**, 551–555.
- Bunikowski, R., Mielke, M.E., Skarabis, H., Worm, M., Anagnostopoulos, I., Kolde, G., Wahn, U. and Renz, H. (2000) Evidence for a disease-promoting effect of *Staphylococcus aureus*-derived exotoxins in atopic dermatitis. *J Allergy Clin Immunol* **105**, 814–819.
- Chen, W.Q., Zhang, M.H. and Bi, Z.G.A. (2005) Study on superantigen production by *Staphylococcus aureus* colonizing in skin lesion and its clinical significance in the patients with atopic dermatitis and eczema. *J Clin Dermatol* **34**, 22–24.
- Cho, S.H., Strickland, I., Boguniewicz, M. and Leung, D.Y. (2001) Fibronectin and fibrinogen contribute to the enhanced binding of *Staphylococcus aureus* to atopic skin. *J Allergy Clin Immunol* **108**, 269–274.
- Cogen, A.L., Nizet, V. and Gallo, R.L. (2008) Skin microbiota: a source of disease or defence? *Br J Dermatol* **158**, 442–455.
- Gloor, M., Peters, G. and Stoika, D. (1982) On the resident aerobic bacterial skin flora in unaffected skin of patients with atopic dermatitis and in healthy controls. *Dermatologica* **164**, 258–265.
- Grice, E.A., Kong, H.H., Conlan, S., Deming, C.B., Davis, J., Young, A.C., Bouffard, G.G., Blakesley, R.W. et al. (2009) Topographical and temporal diversity of the human skin microbiome. *Science* **324**, 1190–1192.
- Hanifin, J.M. and Rajka, G. (1980) Diagnostic features of atopic dermatitis. *Acta Derm-Venereol* **92**, 44–47.
- Hauser, C., Wuethrich, B., Matter, L., Wilhelm, J.A., Sonnabend, W. and Schopfer, K. (1985) *Staphylococcus aureus* skin colonization in atopic dermatitis patients. *Dermatologica* **170**, 35–39.
- Hoeger, P.H., Lenz, W., Boutonnier, A. and Fournier, J.M. (1992) Staphylococcal skin colonization in children with atopic dermatitis: prevalence, persistence, and transmission of toxigenic and nontoxigenic strains. *J Infect Dis* **165**, 1064–1068.
- Hwang, S.Y., Kim, H.K., Jang, E.J., Kwon, N.H., Park, Y.K., Koo, H.C., Jung, W.K., Kim, J.M. et al. (2007) Novel multiplex PCR for the detection of the *Staphylococcus aureus* superantigen and its application to raw meat isolates in Korea. *Int J Food Microbiol* **117**, 99–105.
- Iwase, T., Seki, K., Shinji, H., Mizunoe, Y. and Masuda, S. (2007) Development of a real-time PCR assay for the detection and identification of *Staphylococcus capitis*, *Staphylococcus haemolyticus* and *Staphylococcus warneri*. *J Med Microbiol* **56**, 1346–1349.
- Iwase, T., Uehara, Y., Shinji, H., Tajima, A., Seo, H., Takada, K., Agata, T. and Mizunoe, Y. (2010) *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature* **465**, 346–349.
- Kong, H.H., Oh, J., Deming, C., Conlan, S., Grice, E.A., Beatson, M.A., Nomicos, E., Polley, E.C. et al. (2012) Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res* **22**, 850–859.
- Lai, Y., Villaruz, A.E., Li, M., Cha, D.J., Sturdevant, D.E. and Otto, M. (2007) The human anionic antimicrobial peptide dermcidin induces proteolytic defence mechanisms in staphylococci. *Mol Microbiol* **63**, 497–506.
- Lai, Y., Cogen, A.L., Radek, K.A., Park, H.J., Macleod, D.T., Leightle, A., Ryan, A.F., Di Nardo, A. et al. (2010) Activation of TLR2 by a small molecule produced by *Staphylococcus epidermidis* increases antimicrobial defense against bacterial skin infections. *J Invest Dermatol* **130**, 2211–2221.
- Leung, D.Y. and Bieber, T. (2003) Atopic dermatitis. *Lancet* **361**, 151–160.

- Leyden, J.J., Marples, R.R. and Kligman, A.M. (1974) *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br J Dermatol* **90**, 525–530.
- Li, M., Lai, Y., Villaruz, A.E., Cha, D.J., Sturdevant, D.E. and Otto, M. (2007) Gram-positive three-component antimicrobial peptide-sensing system. *Proc Natl Acad Sci USA* **104**, 9469–9474.
- Lin, Y.-T., Wang, C.-T. and Chiang, B.-L. (2007) Role of bacterial pathogens in atopic dermatitis. *Clin Rev Allergy Immunol* **33**, 167–177.
- Lopes, C., Duarte, A.F., Correia, O. and Delgado, L. (2011) Atopic dermatitis, innate immunity, and infection. *Dermatol Online J* **17**, 1–4.
- Løvseth, A., Loncarevic, S. and Berdal, K.G. (2004) Modified multiplex PCR method for detection of pyrogenic exotoxin genes in staphylococcal isolates. *J Clin Microbiol* **42**, 3869–3872.
- Martineau, F., Picard, F.J., Roy, P.H., Ouellette, M. and Bergeron, M.G. (1996) Species-specific and ubiquitous DNA-based assays for rapid identification of *Staphylococcus epidermidis*. *J Clin Microbiol* **34**, 2888–2893.
- McCrea, K.W., Hartford, O., Davis, S., Eidhin, D.N., Lina, G., Speziale, P., Foster, T.J. and Hook, M. (2000) The serine-aspartate repeat (Sdr) protein family in *Staphylococcus epidermidis*. *Microbiology* **146**, 1535–1546.
- Mehrotra, M., Wang, G. and Johnson, W.M. (2000) Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J Clin Microbiol* **38**, 1032–1035.
- Mempel, M., Lina, G., Hojka, M., Schnopp, C., Seidl, H.-P., Schäfer, T., Ring, J., Vandenesch, F. et al. (2003) High prevalence of superantigens associated with the *egc* locus in *Staphylococcus aureus* isolates from patients with atopic eczema. *Eur J Clin Microbiol* **22**, 306–309.
- Nada, H.A., Gomaa, N.I.M., Elakhras, A., Wasfy, R. and Baker, R.A. (2012) Skin colonization by superantigen-producing *Staphylococcus aureus* in Egyptian patients with atopic dermatitis and its relation to disease severity and serum interleukin-4 level. *Int J Infect Dis* **16**, 29–33.
- Nilsson, M., Frykberg, L., Flock, J.I., Pei, L., Lindberg, M. and Guss, B. (1998) A fibrinogen-binding protein of *Staphylococcus epidermidis*. *Infect Immun* **66**, 2666–2673.
- Nishijima, S., Namura, S., Higashida, T. and Kawai, S. (1997) *Staphylococcus aureus* in the anterior nares and subungual spaces of the hands in atopic dermatitis. *J Int Med Res* **27**, 155–158.
- Ong, P.Y., Ohtake, T., Brandt, C., Strickland, I., Boguniewicz, M., Ganz, T., Gallo, R.L. and Leung, D.Y. (2002) Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *New Engl J Med* **347**, 1151–1160.
- Palmer, C.N., Irvine, A.D., Terron-Kwiatkowski, A., Zhao, Y., Liao, H., Lee, S.P., Goudie, D.R., Sandilands, A. et al. (2006) Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* **38**, 441–446.
- Peschel, A., Jack, R.W., Otto, M., Collins, L.V., Staubitz, P., Nicholson, G., Kalbacher, H., Nieuwenhuizen, W.F. et al. (2001) *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with l-lysine. *J Exp Med* **193**, 1067–1076.
- Roll, A., Cozzio, A., Fischer, B. and Schmid-Grendelmeier, P. (2004) Microbial colonization and atopic dermatitis. *Curr Opin Allergy Clin Immunol* **4**, 373–378.
- Rosenthal, M., Goldberg, D., Aiello, A., Larson, E. and Foxman, B. (2011) Skin microbiota: microbial community structure and its potential association with health and disease. *Infect Genet Evol* **11**, 839–848.
- Sandilands, A., Terron-Kwiatkowski, A., Hull, P.R., O'Regan, G.M., Clayton, T.H., Watson, R.M., Carrick, T., Evans, A.T. et al. (2007) Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* **39**, 650–654.
- Schlievert, P.M., Case, L.C., Strandberg, K.L., Abrams, B.B. and Leung, D.Y. (2008) Superantigen profile of *Staphylococcus aureus* isolates from patients with steroid-resistant atopic dermatitis. *Clin Infect Dis* **46**, 1562–1567.
- Schuencck, R.P., Pereira, E.M., Iorio, N.L. and dos Santos, K.R. (2008) Multiplex PCR assay to identify methicillin-resistant *Staphylococcus haemolyticus*. *FEMS Immunol Med Microbiol* **52**, 431–435.
- Sieprawska-Lupa, M., Mydel, P., Krawczyk, K., Wojcik, K., Puklo, M., Lupa, B., Suder, P., Silberring, J. et al. (2004) Degradation of human antimicrobial peptide LL-37 by *Staphylococcus aureus*-derived proteinases. *Antimicrob Agents Chemother* **48**, 4673–4679.
- Silva, S.H., Guedes, A.C., Gontijo, B., Ramos, A.M., Carmo, L.S., Farias, L.M. and Nicoli, J.R. (2006) Influence of narrow-band UVB phototherapy on cutaneous microbiota of children with atopic dermatitis. *J Eur Acad Dermatol* **20**, 1114–1120.
- Soares, J.C., Marques, M.R., Tavaría, F.K., Pereira, J.O., Malcata, F.X. and Pintado, M.M. (2011) Biodiversity and characterization of *Staphylococcus* species isolated from a small manufacturing dairy plant in Portugal. *Int J Food Microbiol* **146**, 123–129.
- Tavaria, F.K., Soares, J.C., Reis, I.L., Paulo, M.H., Malcata, F.X. and Pintado, M.E. (2012) Chitosan: antimicrobial action upon staphylococci after impregnation onto cotton fabric. *J Appl Microbiol* **112**, 1034–1041.
- Vannuffel, P., Heusterspreute, M., Bouyer, M., Vandercam, B., Philippe, M. and Gala, J.-L. (1999) Molecular characterization of *femA* from *Staphylococcus hominis* and *Staphylococcus saprophyticus*, and *femA*-based discrimination of staphylococcal species. *Res Microbiol* **150**, 129–141.

- Williams, R.J., Henderson, B., Sharp, L.J. and Nair, S.P. (2002) Identification of a fibronectin-binding protein from *Staphylococcus epidermidis*. *Infect Immun* **70**, 6805–6810.
- Yagi, S., Wakaki, N., Ikeda, N., Takagi, Y., Uchida, H., Kato, Y. and Minamino, M. (2004) Presence of staphylococcal exfoliative toxin A in sera of patients with atopic dermatitis. *Clin Exp Allergy* **34**, 984–993.
- Zecconi, A., Cesaris, L., Liandris, E., Daprà, V. and Piccinini, R. (2006) Role of several *Staphylococcus aureus* virulence factors on the inflammatory response in bovine mammary gland. *Microb Pathogenesis* **40**, 177–183.
- Zuel-Fakkar, N.M. and El-Shokry, M.H. (2010) Study of erythroderma and psoriasis exacerbation by staphylococcal superantigens. *J Egypt Women Dermatol Soc* **7**, 123–128.