**Methods**

The study involved 56 adult AD patients with active skin lesions (age 18–70 years, mean 32.8 ± 12.9). All subjects fulfilled the Hanifin and Rajka criteria for AD [1]. The characteristics of the study group are presented in Table 1. The patients provided a written informed consent prior to participation, and their anonymity was fully preserved.

Patients with other skin disorders and with a history of immunosuppressive therapy were excluded. Sporadic, short-term use of antibiotics due to infection over 2 months before the examination was allowed. The routine treatment of AD including emollients, topical corticosteroids, topical calcineurin inhibitors, and oral antihistamines was withdrawn 5 days before the evaluation.

Clinical examination was performed by one investigator (L.B.). Disease severity was determined using the SCORAD index. The maximum extent of skin lesions during AD flares in the last year and during stable periods of the disease was determined based on the Wallace rule of nines [2]. The total IgE serum concentration was measured using the ELISA method (the UniCap Fluorometer).

The propensity to form biofilm by *S. aureus* strains isolated from the anterior nares, lesional skin, and nonlesional skin was individually correlated with constitutional, clinical, and immunological characteristics of the study group.

**Laboratory Determination**

Swabs for microbiological examination were taken from the anterior nares, lesional skin, and nonlesional skin. Swabs from lesional skin were collected from the most intense lesion. Nonlesional skin swabs were routinely taken from the volar forearm and, if that area was involved, from another non-inflamed region of the skin. Swabs were taken with cotton wool-tipped swab sticks immersed in 0.85% NaCl solution (bioMérieux, Marcy l'Etoile, France) and secured in a transport medium (MedLab Products, Raszyn, Poland). Nasal samples were taken by performing clockwise and anti-clockwise 360° turns in both nostrils, and skin samples by rubbing a 4-cm2 (2 × 2 cm) field of the skin for 5 s. Within 24 h the swabs were plated on mannitol-salt agar medium (bioMérieux) and incubated for 24 h under aerobic conditions at 37°C. In case of insufficient growth, the incubation was prolonged up to 48 h. After incubation, all morphologically distinct colonies which caused the yellowing of the medium were isolated and re-inoculated to other plates with mannitol salt so as to obtain a pure laboratory culture [3].

Identification of *S. aureus* was performed by means of the VITEK MS mass spectrometer based on MALDI-TOF (Matrix-Assisted Laser Desorption Ionization Time-of-Flight) technology (bioMérieux) [4].

All identified strains of *S. aureus* were subsequently frozen at –70 °C in brain-heart infusion broth (bioMérieux) for further analyses.

**Analysis of Propensity to Form Biofilm in vitro**

For the analysis of propensity to form biofilm in vitro, *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 35984 were used as the positive controls. The procedure was carried out according to the method presented by Christensen et al. [5] and its subsequent modifications [6, 7].

Defrozen clinical strains were passaged twice on Columbia agar medium with 5% sheep blood (bioMérieux), and a single colony was used to inoculate a culture in TSB (tryptic soy broth) 0.25% glucose medium (bioMérieux) which was subsequently incubated for 20–24 h in aerobic conditions at 37°C. The obtained cultures were used to prepare a bacterial suspension in 0.85% NaCl (0.5 McFarland standard; bioMérieux). The suspensions were diluted 1:100 in sterile TSB 0.25% glucose, and 200 µL was applied to a single well in a 96-well flat-bottom microtiter plate (Profilab S.C., Warsaw, Poland; the procedure was repeated 3 times per strain, i.e., 3 wells were filled with suspension with each strain). A pure TSB 0.25% glucose medium without the bacterial inoculum served as the sterility control for the prepared media. The titer plates were incubated for 24 h in aerobic conditions at 37 °C, after which they were rinsed three times with sterile PBS buffer (AppliChem, Darmstadt, Germany), 200 µL for each well in order to remove bacterial cells unbound in the biofilm. The formed layer of biofilm was dyed with 2% crystal violet solution (bioMérieux) for 15 min, after which the wells were rinsed to the point when the water became colorless. Next, the titer plates were dried for 1 h in 50°C to consolidate the biofilm, and each well was filled with 200 µL of 96% ethanol solution (POCH, Gliwice, Poland). After 3 min, the absorbance at 492 nm wavelength was measured with a STAT FAX 2100 Microplate Reader (Awareness Technology Inc., Palm City, FL, USA). The three readings for each strain were averaged to obtain the final value of absorbance.

The strains for whom the absorbance measurement was: (1) <0.12 were classified as unable to form biofilm (non-slime producers); (2) ≥0.12 and ≤0.24 were classified as capable of weak biofilm formation (weak slime producers); (3) >0.24 were classified as capable of strong biofilm formation (strong slime producers).

**Statistical Analysis**

The frequency table method was used to describe qualitative variables, and a typical measure of position (mean, median) and variability (standard deviation) were used to describe quantitative variables. For selected pairs of variables, associations/correlations were examined. The χ2 test (with the Yates correction) was used to test relationships between categorical variables. In cases when the number of observations was lower than 50, the Fisher test was applied. To compare two groups, the Wilcoxon rank-sum test with continuity correction was used, except attributes with a normal distribution, where the Welch two-sample *t* test was applied. The Spearman rank correlation method was used to measure the degree of the relationship between quantitative and order variables, except in the case of a normal distribution, where the Pearson correlation was applied. To identify a normal distribution, the Shapiro-Wilk normality test was used. For applied methods, the standard level of the *p* value threshold was assumed (*p* < 0.05).

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