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Research paper

Selection of the isolate of *Staphylococcus hominis* for bacteriotherapy in patients with atopic dermatitis

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Abstract

Introduction: *Staphylococcus aureus* plays a significant role in the development of the clinical picture in patients with atopic dermatitis. The use of commensal microflora can be a promising direction in treatment of atopic dermatitis.

Aim: The aim of this study is the selection of the optimal safe isolate of *S. hominis* for bacteriotherapy in patients with atopic dermatitis.

Material and methods: Sensitivity of isolates of *S. hominis* ssp. *hominis* to antibacterial drugs was determined by the disk-diffusion method on Mueller–Hinton agar (HiMedia, India). The ability to form a biofilm was determined by measuring the amount of dye absorption by the biofilm on a microplate reader MR-96A (Mindray, China) at a wavelength of 495 nm. Antagonism in vitro was determined by the method of perpendicular strokes on the blood agar. In total, 24 adult volunteers (aged 18 to 60 years) were screened and included in the study. The results were calculated according to the zone of *S. aureus* growth retardation under the influence of metabolic products of *S. hominis* ssp. *hominis*.

Results and discussion: As a result of the study of 8 isolates of *S. hominis* ssp. hominis, which were obtained from the swabs taken from healthy skin of 24 people, one isolate of *S. hominis* ssp. hominis Hom-2 met all criteria of safety – Hom-2 demonstrated sensitivity to the studied antibiotics and formed a biofilm of low density (OD 0.15), and effectiveness (morphological and cultural properties, antagonistic effect on *S. aureus*).

Conclusions: In this work, an isolate of *S. hominis* ssp. *hominis* Hom-2 met all criteria of safety and efficacy and will be used in the further study of bacteriotherapy in patients with atopic dermatitis.

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1. INTRODUCTION

Atopic dermatitis (AD) is the most common chronic skin disease caused by genetic, immune, and environmental factors. It occurs, as a rule, in early childhood in persons with a hereditary predisposition to atopic diseases, has a chronic relapsing course, age-related features of the localization and morphology of the foci of inflammation.[1–3]

A disturbed epidermal barrier in patients with AD opens the way for the development of pathogenic and opportunistic bacteria on the affected skin. One of the main representatives of such bacteria is *Staphylococcus* spp., in particular *S. aureus*, which plays a significant role in the development of the clinical picture in patients with AD.[4]

Thus, according to studies,[5–7] skin colonization by *S. aureus* is much more common in people with AD than in healthy people. Previous studies have found that laboratory isolates of coagulase-negative *Staphylococcus* (CoNS) species can produce antimicrobial peptides (AMPs).[8,9] AMPs are essential immune defence molecules produced by neutrophils, Paneth cells, mast cells, epithelial cells, and by some normal flora.[10] Because of their direct antimicrobial action, AMPs control the growth of microorganisms that normally reside on epithelial surfaces, a community of microbes referred to as 'microbiome.' It was shown that no subjects were colonized by *S. aureus* if they also had a normal abundance of CoNS bacteria that produced antimicrobial activity, and that the application of antimicrobial CoNS strains to animal or human skin greatly reduced *S. aureus* colonization.

The use of commensal microflora topically in AD may become a new and promising direction in the treatment of AD.[11–13] A recent human trial investigated the safety and potential benefits of *S. hominis*, a commensal CoNS isolated from the skin of healthy individuals, as a topical bacteriotherapy for AD with promising results.[14] Their research showed that isolates of *S. hominis*, which were isolated from healthy human skin, reduced the number of *S. aureus* on the skin of mice and suppressed the expression of the toxin from *S. aureus* (psmα), which promotes the manifestation of inflammatory phenomena. It was shown[15,16] that for the safe use of *S. hominis*, it is necessary to take into account its ability to damage the epidermal barrier, sensitivity to common antibiotic drugs, minimal ability to form a biofilm, as well as the selectivity of inhibition of *S. aureus* compared to the other members of the human skin microbiota.

The *S. hominis* species is divided into two subspecies, *S. hominis* ssp. *hominis* and *S. hominis* ssp. *novobiosepticus*.[17] At the same time, *S. hominis* ssp. *novobiosepticus* is an opportunist which can cause septicemia, particularly in patients with cancer.[18–20]

Biofilms are specific microbial communities that are formed on biotic and abiotic surfaces by the secretion of extracellular polymeric substances that increase the level of adhesion to surfaces.[21] Bacteria inside the biofilm become more resistant to the influence of various exogenous factors, such as antibacterial drugs.[22] Increased stability of biofilms is explained by several factors: (1) different rate of diffusion of substances; (2) the accumulation in the matrix of extracellular enzymes that destroy antibacterial drugs; (3) unavailability of bacteria due to clumping; (4) stable properties of the cells involved in biofilm formation.[23] Biofilm formation is an intra- and interspecies phenomenon that requires dynamic interactions between bacteria in mixed biofilm communities. Interaction between species of biofilm bacteria is carried out through cell-to-cell communication, metabolic interaction, or spatial organization.[24]

2. AIM

Therefore, the aim of our study was to select the optimal safe isolate of *S. hominis* ssp. *hominis* for bacteriotherapy of AD patients.

3. MATERIAL AND METHODS

In total, 24 adult volunteers (aged 18 to 60 years) were screened and included in the study. Inclusion criteria were: age 18–60 years, availability of informed consent. The exclusion criteria were: any dermatologic disease within the past 4 weeks; treatment with topical or systemic corticosteroids within the past 4 weeks; phototherapy within the past 4 weeks; treatment with topical or systemic antibacterial drugs for any dermatologic disease within the past 4 weeks; severe systemic disease or malignant tumours. Surface bacteria were collected from a pre-measured area (5 cm^2) of healthy forearm skin. In order to study the ability of *S. hominis* to inhibit *S. aureus*, *S. aureus* was cultured from the skin of AD patients. The diagnosis of AD was established according to the Hanifin and Rajka criteria.[25] The inclusion criteria were the duration of AD for more than 1 year; degree of severity according to the SCORAD scale 10–60 points, a positive *S. aureus* skin culture. Exclusion criteria were: treatment with systemic corticosteroids within the past 4 weeks, treatment with topical or systemic antibacterial drugs for any other dermatological disease within the past 4 weeks, severe systemic disease, or malignancy.

3.1. Bacteriological examination of the skin

In a previous study we cultured *S. aureus* from the skin of AD patients.[26] For further research, 4 of these isolates (–AD-1, –AD-2, –AD-3, –AD-4) and isolates of *S. hominis* ssp. *hominis* were selected from healthy skin of 24 people of different sexes and ages.

Isolates of *S. hominis* ssp. *hominis* were collected as follows: Skin swabs were collected using a sterile cotton swab pre-moistened with 0.2% TWEEN 20 solution. The swab was applied for 30 s to the flexor (antecubital fossa) surface of the forearm from a pre-measured area (5 cm^2). Swabs with the selected material were immersed in a tube with Amies transport medium and transported to the bacteriological laboratory. Material from the swab was cultured on selective and differential diagnostic nutrient media: 5% blood agar

(BA) (GRASO, Poland), yolk-salt agar (YSA) (HiMedia, India), meat-peptone broth (MPB) (HiMedia, India) with 5% glucose. The inoculated cups were incubated in a thermostat at a temperature of 37°C for 24–48 h. In the absence of growth on plates with BA within 24 h, the incubation was extended to 48 h. Colonies that, according to the results of bacterioscopy, were formed by gram-positive coccal microflora, were selected for further research. The final identification of microorganisms was carried out by using a Vitek 2 compact system (bioMérieux, France).

Sensitivity of isolates of *S. hominis* ssp. *hominis* to antibacterial drugs was determined by the disk-diffusion method on Muller–Hinton agar (HiMedia, India) according to the recommendations of European Committee for Antimicrobial Susceptibility Testing (EUCAST)[27] using the following scheme: Cefoxitin and oxacillin – screening for methicillin resistance; erythromycin and clindamycin – screening for inducible resistance to clindamycin; norfloxacin – screening for resistance to fluoroquinolones.

3.2. Study of phenotypic biofilm-forming ability

100 μL of MPB with 5% glucose (HiMedia, India) and 10 μL of inoculant containing 0.5 McFarland's daily culture of the studied *Staphylococcus* isolates were added in each well of sterile polystyrene plates (Greiner Bio-One GmbH, Germany) (n = 96). Each individual isolate was cultivated in 1 row with 8 wells. Tablets were cultured in a thermostat at a temperature of 37°C for 48 h. The formed biofilm was fixed with a 96% ethanol solution and stained with a saturated aqueous solution of Congo red for 15 minutes. The contents of the wells were pipetted and transferred to a clean plate, where the amount of dye absorption by the biofilm was measured on an MR-96A microplate reader (Mindray, China) at the wavelength of 495 nm. The density of the formed biofilm was determined by measuring the level of dye adsorption by ethanol, which was expressed in units of optical density (OD). It was considered that the strains do not form biofilms if the OD was less than 0.10. The ability to form a biofilm was considered low if the OD was from 0.10 to 0.49; OD – from 0.50 to 1.0 – the average density of the biofilm and the ability to form it; with values above 1.0 – high ability to form biofilm and its high density.

3.3. Studies of antagonistic ability in vitro

Antagonism in vitro was determined by the method of perpendicular strokes on the BA. The results were calculated according to the zone of *S. aureus* growth retardation under the influence of metabolic products of *S. hominis* ssp. *hominis*.

Antagonism within the biofilm in vitro was studied according to the previously described method of studying the biofilm-forming ability, but at the same time, 50 μL of inoculant of two different daily cultures of *S. hominis* ssp. and *S. aureus* were added to each well of the same row.

Statistical processing of the obtained data was carried out using the statistical package IBM SPSS Statistics Base v. 22 and the software EZR v. 1.32 (graphical interface of the R environment, v. 2.13.0). The research database was systematized in the Microsoft Excel editor. Quantitative data were presented as mean ± standard deviation for parametric data. Statistically significant result was considered at $P < 0.05$.

4. RESULTS

In total, 8 isolates of *S. hominis* ssp. *hominis* were selected as a result of examination of smears taken from healthy skin of 24 people of different sexes and ages.

According to the results of the study of sensitivity to antibacterial drugs (Table 1), it was found that isolates Hom-4 and Hom-7 have phenotypic signs of methicillin resistance, and therefore should be excluded from further research. Isolates Hom-5 and Hom-6 are resistant to erythromycin, and isolate Hom-8 is sensitive to erythromycin only at increased exposure.

For the safety of patients who will undergo bacterial therapy for AD, only isolates that showed 100% sensitivity to all screening antibacterial drugs and do not have phenotypic signs of antibiotic resistance factors were selected, namely Hom-1, Hom-2, Hom-3.

Ability to form a biofilm by isolates of *Staphylococcus spp.* was as follows (Table 2): *S. aureus* formed a biofilm of low density (OD 0.34, 0.36, 0.42, 0.48). Among the isolates of *S. hominis* ssp. *hominis*, Hom-2 formed a biofilm of low density (OD 0.15), while the isolates Hom-1 and Hom-3 formed a biofilm of medium density (OD 0.54–0.57) (Figure 1).

Determination of antagonism in vitro was carried out by the method of perpendicular strokes on the BA (Table 3). It was

Table 1. Sensitivity of isolates of *S. hominis* ssp. *hominis* to antibacterial drugs according to EUCAST recommendations.

Isolate	Cefoxitin	Oxacillin	Clindamycin	Erythromycin	Norfloxacin
Hom-1	29S	26S	20S	24S	28S
Hom-2	28S	24S	19S	26S	27S
Hom-3	31S	28S	22S	25S	29S
Hom-4	20R	06R	24S	24S	20S
Hom-5	28S	25S	20S	06R	24S
Hom-6	29S	23S	24S	13R	22S
Hom-7	22R	13R	26S	24S	23S
Hom-8	29S	24S	21S	19I	20S

Comments: S – sensitive; R – resistant; I – sensitive at increased exposure.

Table 2. The density of the formed biofilm of the studied isolates of *Staphylococcus* spp. when stained with Congo red

Isolate	Biofilm density, $n = 8, \lambda = 495$	Ability of isolates to form a biofilm
Hom-1	0.54	medium
Hom-2	0.15	low
Hom-3	0.57	medium
S. aureus–AD-1	0.36	low
S. aureus–AD-2	0.34	low
S. aureus–AD-3	0.48	low
S. aureus–AD-4	0.42	low

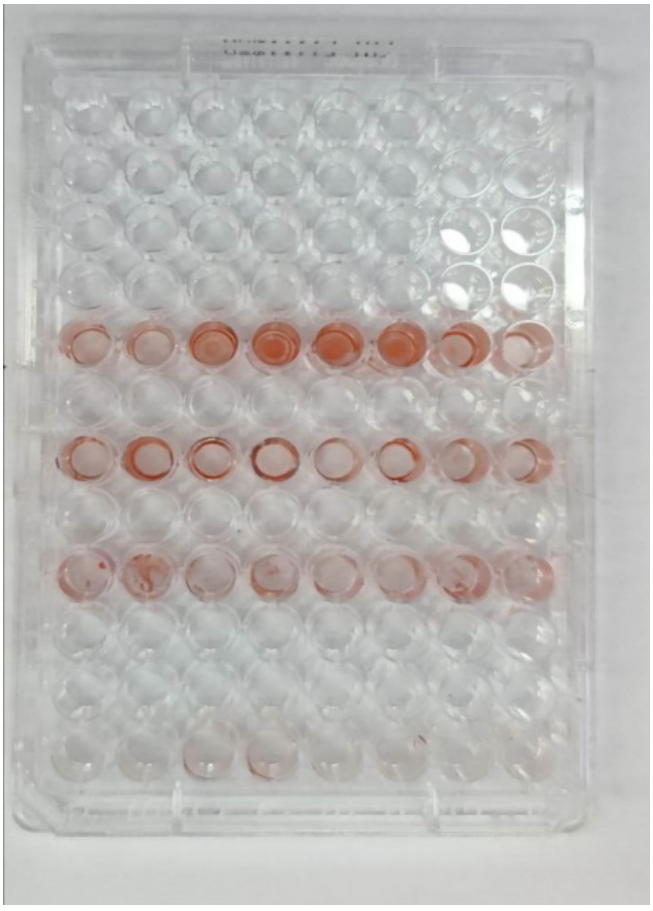

Figure 1. Biofilm formation of *S. hominis* ssp. *hominis*, stained with Congo red (rows from top to bottom Hom-1, Hom-2, Hom-3, respectively).

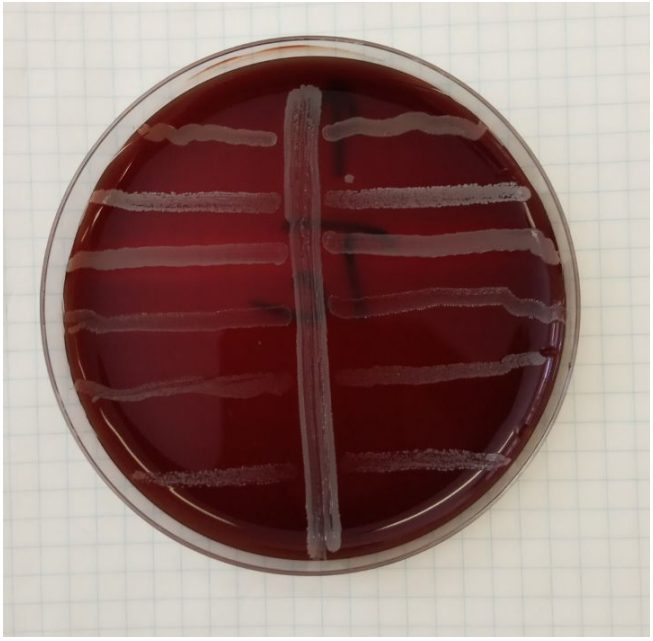

Figure 2. Determination of antagonism of *S. hominis* ssp. *hominis* Hom-3 isolate by the method of perpendicular strokes on the BA.

Table 3. Antagonism of *S. aureus* growth under the influence of metabolic products of *S. hominis* ssp. *hominis*.

Isolate *S. aureus*	Isolate *S. hominis* ssp. *hominis*		
	Hom-1	Hom-2	Hom-3
S. aureus -AD-1	2.0 ± 0.1	3.1 ± 0.1	3.1 ± 0.1
S. aureus-AD-2	2.2 ± 0.1	3.5 ± 0.1	3.8 ± 0.1
S. aureus-AD-3	2.5 ± 0.1	4.2 ± 0.1	5.6 ± 0.1
S. aureus -AD-4	3.0 ± 0.1	4.1 ± 0.1	5.8 ± 0.1

Table 4. Antagonism of *S. hominis* ssp. *hominis* relative to *S. aureus* inside the biofilm when stained with Congo red.

Isolate *S. aureus*	Density of complex biofilm of *S. hominis* ssp. *hominis* Hom-2 $n = 8, \lambda = 495$	Presence of antagonism
S. aureus–AD-1	0.45	lacking
S. aureus–AD-2	0.42	lacking
S. aureus–AD-3	0.58	lacking
S. aureus–AD-4	0.46	lacking

found that isolates of *S. hominis* ssp. *hominis* Hom-1, Hom-2, Hom-3 suppressed the growth of *S. aureus* (2.0–5.8 ± 0.1 mm). It was shown that the isolate of *S. hominis* ssp. *hominis* Hom-3 showed the highest antagonistic activity (5.6 mm) (Figure 2), while isolate Hom-1 (3.0 mm) showed the least antagonistic activity. Hom-2 had average antagonism values (4.2 mm).

Taking into account the data of the preliminary results of the study of the biofilm-forming ability and antagonistic properties of the isolates of *S. hominis* ssp. *hominis*, and data on the safety and effectiveness of the use of bacterial therapy for AD, the Hom-2 isolate was selected. This isolate demonstrated the lowest ability to form a biofilm and sufficient antagonistic capacity against *S. aureus* isolates.

The result of antagonism within the biofilm was taken into account as follows: In the case when the OD of the formed *S. aureus* biofilm together with the Hom-2 isolate was lower than the OD of each individual isolate (Table 2), the ability to antagonize within the biofilm was considered positive. In the case when the OD of the formed complex biofilm was equal to or greater than the OD of each individual isolate, this result was considered as the absence of antagonism of *S. hominis* ssp. *hominis* against *S. aureus* within the biofilm.

OD of phenotypically formed complex biofilms by isolates of *S. hominis* ssp. *hominis* Hom-2 and S. aureus in all cases exceeded the OD of biofilms of each individual isolate (Table 4).

5. DISCUSSION

In this study, a safe isolate of *S. hominis* ssp. *hominis* was selected, which in the future is planned to be used for bacteriotherapy in AD patients.

Isolates that had any phenotypic resistance to antibacterial drugs were culled, since great importance was attributed to

the safety of these isolates for human health. Also, the isolates that had the lowest ability to form a biofilm were excluded, according to the studies.[15,16] In addition, since the ultimate goal is the potential use of bacteriotherapy, we culled *S. hominis* isolates that had unsatisfactory culture properties.

Two methods of phenotypic determination of antagonism were used in this study. The perpendicular stroke method used for the planktonic form demonstrated antagonism between *S. hominis* and *S. aureus*. We evaluated the ability of *S. hominis* isolates to inhibit the growth of *S. aureus* in vitro. *S. hominis* grown on agar produced a distinct zone of inhibition of *S. aureus* growth, thus confirming that this strain spontaneously secreted antagonistic metabolites into the culture medium at a level sufficient to directly inhibit *S. aureus*. At the same time, antagonism of the isolates was not observed under the conditions of biofilm formation in vitro. Since a significant number of factors that promote or, on the contrary, inhibit the ability of microorganisms to adhere and form a biofilm, are not taken into account, this method needs to be refined.

Nakatsuji reported that *S. hominis* produces two potent lantibiotics.[16] These lantibiotics were constitutively secreted by bacteria at concentrations that are sufficient to kill *S. aureus* on the skin surface, and highly synergistic with human AMP LL-37. In the following study, Nakatsuji et al.[14] revealed the mechanisms of the antagonistic action of *S. hominis* cultures on *S. aureus* cultures isolated from the AD patients: The authors isolated the ShA9 strain, based on its capacity to selectively kill and inhibit toxin production by *S. aureus*. Certain strains of *S. epidermidis* can also exacerbate AD, and it was shown that ShA9 also inhibits quorum sensing by *S. epidermidis*.[28] Thus, the authors concluded that *S. hominis* could potentially also act on patients who are *S. aureus* culture negative.

Thus, when developing an effective scheme for bacteriotherapy of AD using a representative of the normal flora of the human skin, it is necessary to conduct a bacteriological study for each individual isolate. The monitoring of antibiotic sensitivity and the ability to form a biofilm of these isolates is equally important to ensure 100% safety of patients undergoing bacterial therapy for AD. As a result of the study of 8 isolates of *S. hominis ssp. hominis*, which were obtained from the swabs taken from healthy skin of 24 people of different sexes and ages, only one isolate of *S. hominis ssp. hominis* Hom-2 met all criteria of safety (resistance to antibacterial drugs, ability to form biofilms) and effectiveness (morphological and cultural properties, antagonistic effect on *S. aureus*).

6. CONCLUSIONS

(1) The use of commensal microflora topically in AD may become a new and promising direction in the treatment of AD.
(2) In this work we have shown that an isolate of *S. hominis* ssp. *hominis* Hom-2 met all the safety and efficacy criteria: Hom-2 demonstrated sensitivity to the studied antibiotics and formed a biofilm of low density (OD 0.15).
(3) The results of this study allow us to continue studying the role of *S. hominis*, a commensal CoNS isolated from the skin of healthy individuals as a topical bacteriotherapy in treatment of AD: Since it has an antagonistic effect on *S. aureus*, probably due to the production of lantibiotics, its displacement on the skin will be achieved in a short period of treatment.

Conflict of interest

The authors declare no conflict of interest.

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Ethics

This study was approved by the ethics committee of the NMU named after O.O. Bogomolets (Protocol No. 2 dated October 21, 2020). Written informed consent was obtained from all participants.

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