ORIGINAL ARTICLE

Features of the skin microbiome in patients with chronic eczema during relapse

ANDRII DASHCHUK[1], MARYNA MISHYNA[2], ANDRII DASHCHUK[1], YEVHENIIA DOBRZHANSKA[1], ALESSANDRA MARCHESE[3], ROBERTA PISI[3], OLHA BONDARENKO[2,3], MARINA AIELLO[3], ALFREDO CHETTA[3]

[1]Department of Dermatology, Venereology and AIDS, Kharkiv National Medical University, Kharkiv, Ukraine; [2]Department of Microbiology, Virology and Immunology named after D.P. Grynyov, Kharkiv National Medical University, Kharkiv, Ukraine; [3] Department of Medicine and Surgery, University of Parma, Parma, Italy

ABSTRACT

Background and Aim: In the presence of eczema, the skin serves as a reservoir for endogenous and exogenous infectious agents. Therefore, in order to understand the mechanism of interaction between microorganism and the macroorganism, it is necessary to study the characteristics of the skin microbiota in individuals with chronic eczema. The aim of this study is to determine the characteristics of changes in the skin microbiome during relapse of chronic eczema depending on age and gender.

Methods: The results of a study of 80 patients with chronic eczema during relapse are presented. The study of the skin microbiome used a culture-based method. Isolated pure cultures of clinical strains were identified using MICRO-LA-TEST® identification kits. Microorganism suspensions with a specific concentration of microbial cells were prepared using a Densi-La-Meter.

Results: Gram-positive microflora, represented by *Staphylococcus spp*. and *Streptococcus spp*., predominated in the affected areas. A significant increase in microbial colonisation density was observed in men aged 61 to 81 years. In the control group, on the contrary, the highest colonisation density was observed in individuals aged 41 to 60 years.

Conclusions: Dysbiotic changes were detected in affected and unaffected skin areas during chronic eczema recurrences across all age groups. It was found that women suffer more often (67.5% of cases) than men. However, the density of microorganism colonisation of skin areas was higher in men, which may be due to

Received: 5 October 2025 | Accepted: 5 November 2025

Correspondence: Olha Bondarenko, PhD / Department of Microbiology, Virology and Immunology named after prof. D.P. Grynyov Kharkiv National Medical University; Department of Medicine and Surgery, University of Parma, G. Rasori Str.,10 Parma, 43125, Italy / E-mail: bondarenkolor84@gmail.com
ORCID: 0000-0002-4808-9513

hormonal differences and the influence of estrogens on the microbiome of the skin and mucous membranes. (www.actabiomedica.it)

Key words: skin, microbiome, eczema, skin lesions, microorganisms

Introduction

The identification of interrelationships among the components of the human body microbiota has enabled specialists to advance to population-level studies of microbial associations in various pathologies, particularly those affecting the skin. However, data on the nature and severity of systemic disturbances in the microbiota composition in chronic eczema, against the background of dysbiotic changes caused by external factors, remain fragmented and unsystematic, characterized by a narrow approach and arbitrary interpretation of various indicators. The study of the human skin microbiota is of particular relevance in the context of socially significant chronic dermatoses, for which there is still no clear understanding of the mechanisms of etiology and pathogenesis. In this context, the skin biotope serves as an example of a complex associative interaction of microorganisms, whose potential etiological significance in the course of chronic eczema should not be underestimated. Eczema accounts for up to 40% of all skin diseases, and among the various clinical forms, microbial eczema occurs in approximately 30% of cases (1, 2). Temporary disability due to eczema reaches up to 36% of all disability cases caused by dermatoses (3, 4). Eczema is considered a polyetiological disease, and the scientific literature indicates that the dominant components of the microbiome in affected skin areas in chronic eczema are gram-positive coccal bacteria, including *Streptococcus pyogenes* with a wide range of serological variants, *Staphylococcus aureus*, and *Staphylococcus epidermidis* (5, 6). In chronic eczema lesions, gram-negative microorganisms such as *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* may also be detected. The number of yeast-like fungi of the genus *Candida spp.* (7, 8) is increasing, and they contribute to the eczematous process as part of mixed infections in more than half of all cases (9, 10). Foci of chronic eczema often develop on the background of postoperative wounds, trophic ulcers, and other skin lesions, particularly in elderly individuals (11, 12). Comorbid background conditions significantly impair the skin's barrier function. The influence of microbial factors in the presence of immunodeficiency leads to the persistence of microbial allergens, sensitization of the body to infectious agents and to the protein components of the skin itself (autosensitization) (13). Thus, the skin microflora, on the one hand, serves as a defence mechanism, and on the other hand, acts as a reservoir of pathogens of exogenous and endogenous infections. Therefore, it forms an ecological barrier that ensures colonization resistance and decontamination of the host organism. It is known that occupational factors negatively affect the microbiome: against the background of dysbiosis, microorganisms acquire increased pathogenicity. However, the role of specific representatives of the skin microbiocenosis in the development and course of chronic eczema remains insufficiently understood. Microflora characteristics are recognised as indicators of the impact of adverse environmental factors on the host organism. However, this indicator is rarely used to assess the impact of harmful occupational factors, and the mechanisms underlying changes in the composition of the skin microbiota in chronic eczema remain poorly understood. Thus, the study of the characteristics of the skin microbiota in patients with chronic eczema under various conditions of onset may serve as one of the key elements in uncovering and understanding the mechanisms of interaction between the micro- and macroorganism in this pathology. The aim of this study is to investigate the characteristics of skin

microbiome changes during relapse of chronic eczema depending on age and sex.

Patients and methods

The primary study group consisted of 80 patients with chronic eczema, including 54 women and 26 men, aged 24 to 81 years, without severe somatic or infectious comorbidities. The control group included 20 individuals. Inclusion criteria: informed consent to participate in the study; compliance with medical recommendations; confirmed diagnosis of chronic eczema. Exclusion criteria: history of biological therapy; presence of severe somatic or infectious comorbidities; use of systemic corticosteroids within 6 months before the start of the study. The study of skin microbiocenosis was conducted using a culture-based method. Sample collection and bacteriological examination were performed according to generally accepted techniques (14). To isolate pure cultures, the following recommended nutrient media were used: 5% blood agar, serum agar, serum broth, Endo medium, yolk-salt agar, Hugh–Leifson medium (OF-test), Mueller–Hinton agar, and Sabouraud medium. Isolated pure cultures of clinical strains were identified using MICRO-LA-TEST® identification kits. Suspensions of microorganisms with a specific microbial cell concentration were prepared according to the McFarland standard using a Densi-La-Meter (PLIVA-Lachema a.s., Czech Republic), following the manufacturer's instructions: for *Enterobacteriaceae*, the turbidity of the suspension corresponded to McFarland standard 1; for *Staphylococci* and non-fermenting bacteria – standard 2; and for *Streptococci* and *Fungi* – standard 3. Statistical analysis of the study results was performed using the StatSoft "Statistica 7" software package, with the reliability of the obtained values determined using Student's t-test (15, 16). This study was conducted in accordance with the ethical standards and current legislation of Ukraine, as confirmed by the Ethics and Bioethics Committee of Kharkiv National Medical University (excerpt from protocol № 22 dated October 3, 2024). The research was carried out within the framework of the scientific project "Optimization of prevention and therapy of secondary infectious complications in dysbiotic patients with chronic dermatoses", state registration (№ 0122U200326, conducted from 2022 to 2024).

Results

The patients ages ranged from 24 to 81 years. Notably, among the examined patients, chronic eczema was most frequently observed in individuals aged 41–61 years (47.5%). In elderly individuals (aged 61–81), the prevalence of eczema was 32.5%, while in the 24–40 age group it accounted for 20% of cases. In all age groups, women were affected more often (67.5%) than men (32.5%). During the examination of healthy individuals, the normal skin microbiota was found to be composed of a variety of microorganisms. The skin microbial consortium included representatives of gram-positive flora, with the largest group comprising *Staphylococcus spp*. Among the conditionally pathogenic microorganisms, *Staphylococcus epidermidis* strains were most frequently detected, with the highest colonization density observed in men aged 41–60 years (51.9±2.6 CFU/cm²). *Staphylococcus sciuri, Staphylococcus xylosus, Staphylococcus simulans, Micrococcus luteus*, and *Enterococcus faecium* were identified at low frequencies and low colonization densities, and were entirely absent in the 61–81 age group regardless of gender. It is worth noting that *Acinetobacter* species are present in the normal microbiota only in women aged 24–40 and 41–60 years. *Enterococcus faecalis* was also present in the skin microbiota of the examined individuals, with colonization densities ranging from 18.6±1.8 CFU/cm² in women aged 61–81 years to 43.9±2.4 CFU/cm² in men aged 41–60 years. In addition to coccal microorganisms, *Corynebacterium spp.* formed a large group (Table 1).

Analysis of the skin microbiocenosis in affected and unaffected areas in patients with chronic eczema during relapse revealed a significantly higher density of staphylococcal colonization in the affected skin regions across all study groups, compared to both the adjacent unaffected skin ($p<0.05$) and the control group values ($p<0.001$). The average density of *S. aureus* colonization in affected skin areas in patients with chronic eczema aged 24–40 years (Table 2) was 490.9 ± 4.9 CFU/cm²

Table 1. Skin microbiome of the control group (n=20) depending on age and sex (M±m, CFU/cm²).

№	Type of microorganism	24-40 years (n=6)		41-60 years (n=8)		61-81 years (n=6)	
		F (n=3)	M (n=3)	F (n=4)	M (n=4)	F (n=3)	M (n=3)
1	*S. aureus*	32,6±1,2	39,2±1,8	42,4±2,2	48,1±2,4	21,8±1,6	28,4±1,2
2	*S. epidermidis*	42,6±2,8	48,4±2,2	49,2±2,4	51,9±2,6	31,4±2,2	39,2±2,1
3	*S. saprophyticus*	11,8±1,2	14,3±1,1	16,1±1,3	18,2±1,9	8,6±1,4	10,1±1,1
4	*S. haemolyticus*	6,2±1,6	7,6±1,2	8,6±1,2	9,8±1,3	4,8±1,2	6,2±1,4
5	*S. warneri*	3,6±0,2	4,2±0,2	4,5±0,5	5,9±0,8	2,9±0,8	3,4±0,6
6	*S. hominis*	6,2±1,2	8,4±1,6	9,8±1,6	10,6±1,2	4,1±1,3	6,2±1,8
7	*S. capitis*	5,3±1,1	6,1±1,2	7,2±1,4	8,5±1,5	3,5±1,5	4,8±1,6
8	*S. sciuri*	1,2±0,2	1,8±0,6	1,9±0,6	2,3±0,9	-	-
9	*S. xylosus*	0,1±0,01	0,6±0,02	1,1±0,1	1,4±0,6	-	-
10	*S. simulans*	0,2±0,02	0,9±0,3	1,4±0,2	1,8±0,4	-	-
11	*M. luteus*	0,1±0,02	0,3±0,01	0,9±0,06	1,1±0,8	-	-
12	*E. faecium*	0,6±0,02	1,1±0,08	1,3±0,1	1,9±0,6	-	-
13	*E. faecalis*	31,2±1,4	36,1±1,7	38,8±1,9	43,9±2,4	18,6±1,8	24,2±1,4
14	*Acinetobacter spp.*	0,6±0,2	-	1,2±0,4	-	-	-
15	*Corynebacterium spp.*	22,9±1,8	28,4±1,2	32,4±1,2	36,2±1,4	9,8±0,6	16,2±0,8
16	*K. pneumoniae*	-	-	-	-	-	-
17	*Streptococcus spp.*	41,2±2,4	47,4±2,6	51,5±2,5	58,3±2,1	29,8±1,2	38,2±1,8
18	*C. albicans*	0,6±0,04	1,1±0,02	1,2±0,4	1,6±0,8	-	-
19	*P. aeruginosa*	-	-	-	-	-	-
20	*Proteus spp.*	-	-	-	-	-	-

Table 2. Structure of the skin microbial consortium in patients aged 24–40 years (n=16) with recurrent chronic eczema (M±m, CFU/cm²).

№	Type of microorganism	Sex			
		Female (n=10)		Male (n=6)	
		Age group 24-40 years (n=16)			
		affected areas	intact areas	affected areas	intact areas
1	*S. aureus*	348,2±4,1*#	133,6±3,6*	490,9±4,9*#	179,6±3,6*
2	*S. epidermidis*	285,6±3,6#	97,2±2,3	319,1±4,5*#	103,6±2,3*
3	*S. saprophyticus*	66,4±2,5*	22,4±1,3	84,5±2,4*	43,2±2,1*
4	*S. haemolyticus*	90,2±2,8*#	33,5±1,4	112,3±2,7*#	48,4±2,4*
5	*S. warneri*	157,4±3,5*#	64,5±3,8*	198,2±4,5*#	92,5±3,8*
6	*S. hominis*	98,3±2,9*	25,4±1,7	122,7±3,2*#	48,2±2,7*
7	*S. capitis*	58,1±1,6*	24,3±1,6	107,2±2,8*	47,4±2,6*
8	*S. sciuri*	63,1±3,2*	32,2±1,8	94,8±2,1*	48,6±2,8
9	*S. xylosus*	57,1±1,5#	16,2±1,6	88,2±2,4#	20,4±1,6
10	*S. simulans*	592,4±4,8*#	123,9±3,1*	631,2±4,6*#	154,1±3,7*

№	Type of microorganism	Sex			
		Female (n=10)		Male (n=6)	
		Age group 24-40 years (n=16)			
		affected areas	intact areas	affected areas	intact areas
11	*M. luteus*	150,1±4,3*#	42,3±2,4	192,7±3,8*#	61,8±2,4*
12	*E. faecium*	62,6±2,8	23,9±1,4	109,2±2,6*	42,5±2,5
13	*E. faecalis*	624,2±4,2*#	92,2±2,6	838,8±4,2*#	126,4±2,8
14	*Acinetobacter spp.*	436,8±4,6*#	34,9±2,8	632,3±4,1*#	42,3±2,9
15	*Corynebacterium spp.*	85±4,7**	16,8±1,6	102,5±3,5*#	26,4±1,2
16	*K. pneumoniae*	10,9±1,1*	1,6±0,2	14,6±1,2*	2,9±0,8
17	*Streptococcus spp.*	96,2±2,8**	24,3±1,7	159,2±2,4*#	36,8±1,4
18	*C. albicans*	37,6±2,4*	8,6±0,8	48,9±2,7*	11,8±0,9
19	*P. aeruginosa*	21,1±1,3*	1,2±0,2	28,6±1,4*	1,4±0,2
20	*Proteus spp.*	18,2±1,4*	6,2±0,6	24,9±1,8*	9,6±0,8

Note: differences compared to the control group and contamination levels of intact skin areas are statistically significant: * p<0.001; # p<0.05.

in males and 348.2 ± 4.1 CFU/cm² in females, which was significantly higher compared to the corresponding values in adjacent unaffected skin areas ($p < 0.05$). A predominance of certain *Staphylococcus spp.*, such as *S. epidermidis*, *S. warneri*, and *S. simulans*, was observed over other coccal microorganisms within the skin microbiocenosis. Among the coccal flora, *Micrococcus luteus* was also detected, with a total colonization density of 192.7 ± 3.8 CFU/cm² in males and 150.1 ± 4.3 CFU/cm² in females, which exceeded the colonization density in adjacent unaffected skin by 3.1 and 3.6 times, respectively ($p < 0.05$). A high colonization density was also noted for *Enterococcus faecalis*: 838.8 ± 4.2 CFU/cm² in males and 624.2 ± 4.2 CFU/cm² in females. Among the gram-negative microorganisms, *Pseudomonas aeruginosa* demonstrated the highest colonization density in affected skin areas (28.6 ± 1.4 CFU/cm² in males and 21.1 ± 1.3 CFU/cm² in females), followed by bacteria of the genus *Proteus spp.* (24.9 ± 1.8 CFU/cm² and 18.2 ± 1.4 CFU/cm², respectively).

The obtained results regarding the composition of skin microflora in patients with the chronic eczema during relapse indicate a significant difference between the microbial composition of affected skin areas and that of the adjacent intact skin near the demarcation line, as well as the control group, in patients aged 41–60 years (Table 3). The skin microbiota in chronic eczema is characterized by an increased proportion of coccal flora representatives within the microbiocenosis of the affected skin, along with a high level of microbial colonization density. A notable rise in the significance of *Staphylococcus spp.*, particularly *Staphylococcus aureus*, was observed. It is noteworthy that the colonization density of microorganisms on even the intact skin areas in this age group was higher than in the 24–40-year age group. The colonization density values of intact skin compared to affected areas varied: a high level of colonization of intact skin was recorded for *Staphylococcus epidermidis* 163.4 ± 2.6 CFU/cm² in men and 108.4 ± 2.8 CFU/cm² in women; *Staphylococcus simulans* demonstrated moderate colonization 199.7 ± 3.6 CFU/cm² in men and 158.4 ± 3.8 CFU/cm² in women. On affected areas, however, the colonization density of these microorganisms increased by 1.7 times in men and 2.3 times in women for *Staphylococcus epidermidis*, and by 4.5 times in men and 4.6 times in women for *Staphylococcus simulans*, respectively. Notably, the colonization density of *Enterococcus faecium* was lower compared to *Enterococcus faecalis* on the affected skin areas: 318.3 ± 2.1 CFU/cm² and 905.1 ± 4.9 CFU/cm² in men, and 224.3 ± 2.9 CFU/cm² and 834.6 ± 4.4 CFU/cm² in women, respectively. Among representatives of the gram-negative

Table 3. Structure of the skin microbial consortium in patients aged 41–60 years (n = 38) with recurrent chronic eczema (M ± m, CFU/cm²).

№	Type of microorganism	Sex			
		Female (n=26)		Male (n=12)	
		Age group 41-60 years (n=38)			
		affected areas	intact areas	affected areas	intact areas
1	*S. aureus*	721,9±4,8*#	163,2±3,4*	829,4±4,6*#	198,4±3,8*
2	*S. epidermidis*	252,2±4,4*	108,4±2,8	284,6±4,2*	163,4±2,6
3	*S. saprophyticus*	114,6±3,4*#	43,4±1,9	132,1±3,7*#	68,2±2,4*
4	*S. haemolyticus*	128,4±3,2*#	49,2±1,6	149,4±3,8*#	76,3±2,9*
5	*S. warneri*	204,1±4,3*#	86,4±2,2*	248,1±4,3*	109,2±3,6*
6	*S. hominis*	122,5±3,5*#	65,4±2,8*	192,5±3,2*#	89,3±2,9*
7	*S. capitis*	167,1±3,9*#	62,9±2,3*	217,2±2,8*#	92,1±2,3*
8	*S. sciuri*	127,8±2,2#	48,2±1,4	167,4±2,6*	87,2±2,4*
9	*S. xylosus*	108,4±1,6*	29,2±1,8	129,2±2,8*#	38,1±1,9
10	*S. simulans*	724,2±4,6*#	158,4±3,8*	896,8±4,2*#	199,7±3,6*
11	*M. luteus*	242,5±4,5*	61,4±2,2	329,2±3,6*	89,4±2,2*
12	*E. faecium*	224,3±2,9*#	45,6±1,2	318,3±2,1*#	68,4±2,8*
13	*E. faecalis*	834,6±4,4*#	115,4±2,8#	905,1±4,9*#	191,7±2,3*
14	*Acinetobacter*	618,2±4,8*#	56,2±2,6*	782,1±4,3*	84,6±2,2*
15	*Corynebacterium spp.*	109,6±4,2*#	29,4±1,8	161,4±3,8*	47,2±1,4
16	*K. pneumoniae*	19,1±1,7	1,9±0,3	26,2±1,6	3,7±0,9
17	*Streptococcus spp.*	165,3±2,9*#	32,6±1,8	224,4±2,8*#	49,2±1,6
18	*C. albicans*	61,9±2,1	12,3±0,7	85,6±2,2#	19,1±0,7
19	*P. aeruginosa*	29,8±1,6	1,9±0,3	36,4±1,6	2,8±0,8
20	*Proteus spp.*	26,7±1,3	9,1±0,7	39,8±1,4	11,2±0,6

Note: Differences compared to the control group and contamination levels of intact skin areas are statistically significant: *p < 0.001; # p < 0.05.

microflora, a significant increase in the density of *Acinetobacter* spp. was observed: 782.1 ± 4.3 CFU/cm² in men and 618.2 ± 4.8 CFU/cm² in women, which significantly exceeded the corresponding values on intact skin areas by 9.3-fold and 11-fold, respectively ($p < 0.05$).

When analyzing the obtained results of the skin microbiome in patients aged 61–81 years (Table 4) with chronic eczema during relapse, it is important to note that *Staphylococcus aureus* was most frequently isolated from the patients' skin. The affected areas showed the highest levels of colonization: 968.2 ± 6.8 CFU/cm² in men and 805.4 ± 5.2 CFU/cm² in women ($p < 0.001$), which exceeded the values in the control group by 34.1 times in men and 36.9 times in women. In intact skin areas bordering the eczematous lesions, the colonization density was 207.8 ± 3.6 CFU/cm² in men and 195.1 ± 3.7 CFU/cm² in women, which was 7.3 and 8.9 times higher than in the control group, respectively. The colonization density of *Staphylococcus epidermidis* in the affected skin areas of this study group was 421.3 ± 4.7 CFU/cm² in men and 347.1 ± 4.9 CFU/cm² in women ($p < 0.001$). In intact skin areas, the colonization was 208.6 ± 3.8 CFU/cm² in men and 189.4 ± 4.1 CFU/cm² in women ($p < 0.05$). The density of *Staphylococcus haemolyticus* colonization in the affected skin area in men was twice as high as on the intact skin, and for women - 2.2 times higher. Compared to the control group, these values were 36.8 times higher in men and 34.5 times higher in women

Table 4. Structure of the skin microbial consortium in patients aged 61–81 years (n=26) with recurrent chronic eczema (M±m, CFU/cm²).

№	Type of microorganism	Sex			
		Female (n=18)		Male (n=8)	
		Age group 61-81 years (n=26)			
		affected areas	intact areas	affected areas	intact areas
1	*S. aureus*	805,4±5,2*#	195,1±3,7*	968,2±6,8*#	207,8±3,6*
2	*S. epidermidis*	347,1±4,9*	122,7±2,1 #	421,3±4,7*	194,2±2,8*
3	*S. saprophyticus*	178,3±3,7*#	88,6±2,2*	207,2±3,6*	102,4±3,2*
4	*S. haemolyticus*	165,8±3,6*#	74,6±2,8	228,4±3,8**	112,1±3,3*
5	*S. warneri*	286,4±4,8*#	99,2±3,4	305,1±4,9*	135,6±3,8*
6	*S. hominis*	209,2±4,5*#	108,1±2,7 #	285,6±4,8*	163,1±3,7*
7	*S. capitis*	252,7±4,1*#	89,4±2,8*	395,4±4,6*	109,6±3,8*
8	*S. sciuri*	186,2±3,8*	66,6±2,2*	291,2±4,2*	103,1±3,4 #
9	*S. xylosus*	193,1±3,7*#	48,1±1,9	246,2±4,6*#	76,4±1,6
10	*S. simulans*	896,3±5,9*#	186,2±3,4*	952,3±6,1*#	221,6±4,2*
11	*M. luteus*	351,2±4,4*	96,7±2,9	456,8±4,2*	112,2±3,8*
12	*E. faecium*	388,6±4,6*#	61,6±2,8	502,7±4,1*	96,1±2,7*
13	*E. faecalis*	895,5±5,5*#	181,2±3,6	986,4±6,2*#	216,8±4,2*
14	*Acinetobacter spp.*	735,2±4,6*#	87,1±2,9*	892,6±5,8*	108,1±3,9*
15	*Corynebacterium spp.*	162,1±3,7*#	57,4±2,2	248,2±3,6*	96,4±2,8*
16	*K. pneumoniae*	27,1±1,9*	2,8±0,6	42,1±1,7**	4,9±0,8
17	*Streptococcus spp.*	218,9±3,8*#	54,6±1,2	337,2±4,6**	86,1±2,7*
18	*C. albicans*	91,3±2,9*	28,2±1,4	149,4±3,8*#	48,9±1,8
19	*P. aeruginosa*	34,2±1,4	2,4±0,8	42,1±1,9	4,1±0,9
20	*Proteus spp.*	41,3±1,7	12,6±0,2	61,2±1,4	19,8±0,6

Note: Differences compared to the control group and contamination levels of intact skin areas are statistically significant: * p<0.001; # p<0.05.

($p < 0.001$). Similar changes were observed among bacteria of other species. Thus, *Micrococcus luteus* was most frequently detected in the affected skin area: 456.8±4.2 CFU/cm² in men and 351.2±4.4 CFU/cm² in women, while this species was not identified in the control group. The colonization density of *Streptococcus spp.* in patients of this age group in the affected area was 3.9 times higher in men and 4 times higher in women compared to the intact skin area, reaching 337.2±4.6 CFU/cm² in men and 218.9±3.8 CFU/cm² in women ($p<0.05$). Compared to the control group, this indicator increased 8.8 times in men and 7.3 times in women, respectively ($p<0.001$). The colonization intensity of *Enterococcus faecalis* and *Enterococcus faecium* species also increased in the affected areas: in men 986.4±6.2 CFU/cm^2 and 502.7±4.1 CFU/cm^2 and in women 895.5±5.5 CFU/cm^2 and 388.6±4.6 CFU/cm^2, respectively. In the control, only *Enterococcus faecalis* was detected, the colonization density of which was: in men 24.2±1.4 CFU/cm^2 and in women 18.6±1.8 CFU/cm^2.

Thus, in individuals suffering from chronic eczema during relapse, an increased colonization density by both gram-positive and gram-negative microorganisms was observed on both affected and intact areas of the skin. The intensity of colonization by gram-positive microorganisms was higher than that of gram-negative ones. The results obtained from the examination of the skin of patients and healthy individuals during the conducted study are consistent with the literature data

and indicate significant diversity in the composition of the microbiota and colonization density. The most important characteristics of the bacterial population include both qualitative and quantitative criteria: the number and constant presence of microbes in the biocenosis, that is, the level of contamination of the studied skin biotope (both the lesion site and the intact area adjacent to the eczematous zone). A comparative analysis of the quantitative composition of the microbiota in intact and affected skin areas of patients with chronic eczema revealed significant differences in this parameter, including various *Staphylococcus spp.* occupied a dominant position in the skin microbiocenosis during relapse of chronic eczema, representing the most numerous group of gram-positive microorganisms in terms of both detection frequency and colonization density.

Discussion

Our study revealed significant alterations in the composition and density of skin microbiota in patients with chronic eczema during disease relapse. Both lesional and adjacent intact skin areas exhibited increased colonization, predominantly by gram-positive coccal microflora (*S. aureus, S. epidermidis, Streptococcus spp*.), as well as gram-negative microorganisms (*Proteus spp., P. aeruginosa, Acinetobacter spp.*). These findings highlight the complexity of microbial shifts in chronic dermatoses and are consistent with previously published data on dysbiosis in chronic eczema. Our results align with those of Williams et al., who described *Staphylococcus aureus* as a "master manipulator" of the skin microenvironment, capable of inducing inflammation, disrupting barrier function, and persisting despite the host immune response (5). Excessive growth of *S. aureus* is frequently observed in patients with eczema and is associated with disease flares and poor therapeutic outcomes. Similar results were reported by Nørreslet et al., who found that increased *S. aureus* colonization positively correlated with eczema severity (13). In our cohort, the highest colonization density was recorded in males aged 61 to 81 years. This may be attributed to age-related changes in the skin, the presence of comorbidities, reduced regenerative capacity, and diminished immune responses. In contrast, in the control group, the highest microbial burden was observed in the 41- 60 age category, suggesting a significant decline in colonization resistance in older individuals. Previous studies have indicated that ageing skin becomes more susceptible to microbial imbalance and colonization by opportunistic pathogens (7, 8). A novel aspect of our study is the analysis of sex-related differences. Although eczema was more prevalent among females (67.5%), males demonstrated significantly higher microbial colonization density on both affected and unaffected skin areas. This may be explained by hormonal differences, particularly the protective role of estrogens in promoting a more stable and diverse microbiome, whereas androgens may favor the growth of opportunistic microorganisms (8, 13). The role of gram-negative bacteria in eczema has been less extensively studied. However, in our study, a notable increase in colonization by *Pseudomonas aeruginosa*, *Proteus spp.*, and *Klebsiella pneumoniae* was observed in lesional areas, especially in elderly males. These bacteria produce proteases and other virulence factors that exacerbate epithelial damage and impair healing. This supports the findings of Byrd et al., who noted that although gram-negative species are less abundant, they may dominate when skin barrier integrity is compromised (7). Of particular interest is the high colonization density of *E. faecalis* and *E. faecium* in eczematous lesions. While these microorganisms are typically intestinal commensals, they are increasingly recognized as pathogens in chronic skin conditions. Their presence may indicate translocation from other body sites or secondary infection in the context of compromised skin defenses. From a clinical standpoint, our findings underscore the importance of microbiological evaluation in patients with chronic eczema, especially in cases of poor treatment response or frequent relapses. Although broad-spectrum antibiotics are commonly used, the identification of specific, highly colonising microbial species may serve as a rationale for individualised treatment approaches (e.g., topical probiotics, bacteriophages, targeted antimicrobials). The comparative analysis of lesional and non-lesional skin also deserves attention. Despite lower colonization density in unaffected skin, it significantly exceeded that of the control group, indicating the presence of a subclinical

dysbiotic zone that may act as a reservoir for reinfection. Thus, our data support the contemporary concept that the skin microbiome should be viewed not only as a marker but also as an active participant in disease pathogenesis. Studies by Berg et al. and Nørreslet et al. further substantiate the ecological and immunological role of the microbiome in maintaining skin homeostasis (6,8). Our study contributes to the existing body of knowledge by providing quantitative data stratified by age, sex, and anatomical location, thereby enhancing the understanding of dysbiotic changes in chronic eczema.

Study limitations

The microbiological analysis in our study used culture-dependent methods, which do not capture the full diversity of the skin microbiome. Future research employing molecular techniques such as 16S rRNA sequencing may provide more detailed insights into the microbial composition.

Future directions

Given the patterns observed, future studies should focus on elucidating the immunological and molecular mechanisms through which the microbiota influences the course of chronic eczema. Longitudinal monitoring of microbiome changes during remission and relapse phases may help identify prognostic markers and novel therapeutic targets for effective management of chronic eczema.

Conclusions

Dysbiotic changes were identified in both affected and intact skin areas during chronic eczema relapse across all age groups. The most pronounced alterations observed in men aged 61 to 80 years compared to the corresponding indicators in the control group. A significant increase in colonization density was observed in men aged 61 to 81 years with recurrent chronic eczema, whereas in the control group, the highest colonization density was recorded in individuals aged 41 to 60 years, and the lowest - in the 61 to 81-year-old group. This may be associated with the active professional lifestyle typical for middle-aged men. It was found that women are more frequently affected (67.5% of cases) than men. However, the colonization density of microorganisms in both affected and intact skin areas was higher in men, which may be linked to hormonal differences and the influence of estrogens on the microbiome of the skin and mucous membranes.

Ethic Approval: A Local Ethics Committee of the Department of Microbiology, Virology and Immunology named after D.P.Grynyov, Kharkiv National Medical University approved the study (Protocol № 22, 03/10/2024).

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Authors Contribution: MM, AD: conceptualization, methodology, investigation, resources, formal analysis, writing – original/draft preparation. AD, YD: resources, software, investigation, data curation, supervision. MA, AC, RP: methodology, investigation, formal analysis, validation, writing - review and editing. OB, AM: writing – original/draft preparation, supervision, project administration, methodology.

Declaration on the Use of AI: None.

References

1. Quaade AS, Simonsen AB, Halling AS, Thyssen JP, Johansen JD. Prevalence, incidence and severity of hand eczema in the general population - a systematic review and meta-analysis. Contact Dermatitis 2021;84:361-74. doi:10.1111/cod.13804.
2. Agner T, Elsner P. Hand eczema: epidemiology, prognosis and prevention. J. Eur Acad Dermatol Venereol 2020;34: 4-12. doi:10.1111 /jdv.16061.
3. Mernelius S, Carlsson E, Henricson J et al. Staphylococcus aureus colonization related to severity of hand eczema. Eur J Clin Microbiol Infect Dis. 2016;35(8):1355-61. doi: 10.1007/s10096-016-2672-2.
4. Norreslet LB, Edslev SM, Clausen ML, et al. Hand eczema and temporal variation of Staphylococcus aureus

clonal complexes: a prospective observational study. J Am Acad Dermatol. 2022;87(5):1006-13. doi:10.1016/j.jaad.2021.04.037.
5. Williams MR, Nakatsuji T, Gallo RL. Staphylococcus aureus: master manipulator of the skin. Cell Host Microbe. 2017;22(5):579-81. doi:10.1016/j.chom.2017.10.015.
6. Berg G, Rybakova D, Fischer D, et al. Microbiome definition re-visited: old concepts and new challenges. Microbiome. 2020;8(1):103. doi:10.1186/s40168-020-00875-0.
7. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. Nat Rev Microbiol. 2018;16(3):143-55. doi:10.1038/nrmicro.2017.157.
8. Norreslet LB, Agner T, Clausen ML. The skin microbiome in inflammatory skin diseases. Curr Dermatol Rep. 2020;9:141-51. doi:10.1007/s13671-020-00297-z.
9. Diepgen TL, Andersen KE, Chosidow O, et al. Guidelines for diagnosis, prevention and treatment of hand eczema. J Dtsch Dermatol Ges. 2015;13:e1-22. doi:10.1111/ddg.12510.
10. Agner T, Aalto-Korte K, Andersen KE, et al. Classification of hand eczema. J Eur Acad Dermatol Venereol. 2015;29:2417-22. doi:10.1111/jdv.13308.
11. Held E, Skoet R, Johansen JD, Agner T. The hand eczema severity index (HECSI): a scoring system for clinical assessment of hand eczema. A study of inter- and intraobserver reliability. Br J Dermatol 2005;152:302-7. doi:10.1111/j.1365-2133.2004.06305.x.
12. Kong HH. Details matter: designing skin microbiome studies. J Invest Dermatol 2016;136: 900-2. doi:10.1016/j.jid.2016.03.004.
13. Nørreslet LB, Lilje B, Ingham AC, et al. Skin microbiome in patients with hand eczema and healthy controls: a three-week prospective study. Acta Derm Venereol. 2022;18:102:adv00633. doi:10.2340/actadv.v101.845.
14. Babych YM, Kalinichenko SV, Skliar MI, Myronenko L.H., Peretiatko O.H., Ryzhkova T.A. Preparation of microorganism suspensions with a defined concentration of microbial cells. Laboratory Diagnostics. 2007;3(41):58–61.
15. Strakhova OP. Statistical methods of processing the results of medical and biological research: teaching-methodical manual. Lviv: Marchenko TV; 2023. pp.164.
16. Petrovska I, Saliga Yu, Vudmaska I. Statistical methods in biological research: a teaching and methodological manual. Kyiv:Agrarna nauka; 2022. pp.172.