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Bacterial colonization in atopic dermatitis

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ABSTRACT

Background: Atopic dermatitis (AD) is the most common skin disease in infants and children, influenced by hereditary and environmental factors and characterized by an inflammatory reaction in the skin. The increase in AD has been explained by two hypotheses: the "hygiene hypothesis" and the "diet–microbiome hypothesis." AD is driven by a familial or personal predisposition to induce immunoglobulin E (IgE) antibodies and sensitize in response to stimuli. Microorganisms on the skin have been shown to play an important role in the pathogenesis of AD, and changes in the composition of the skin microbiome have been investigated in disease progression in pediatric AD patients.
Method: This descriptive observational study with a cross-sectional approach aims to determine the bacteria found in AD. The samples were taken from both the lesion and non-lesion areas in children with AD, which grown anaerobically and aerobically in blood and Brucella agar at 37C. The bacteri then indentified by Vitek® 2 and the data presented in percentage.
Result: Thirty-five pediatric patients (18 males and 17 females) with a mean age of 7.58 (0,17 – 16) years were diagnosed with AD and were examined for bacterial culture on lesions and non-lesions. A total of 4 types of bacteria were found on the neck, 20 on the forearm, 2 on hand folds, 2 on fingers, 1 on the knee, and 5 on the lower limbs.
Conclusion: The most common bacteria were Gram-negative bacilli, namely, *Acinetobacter baumannii* (15%) and *Burkholderia cepacian* (14.3%), followed by the Gram-positive coccus *Staphylococcus hominis* (11.4%).

Keywords: Atopic dermatitis, identification of bacteria, pediatric.
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INTRODUCTION

Atopic dermatitis (AD) is a skin disease often found in infants and children, characterized by an inflammatory skin reaction and influenced by hereditary and environmental factors. This chronic recidivism disease has symptoms of erythema, papules, vesicles, crusts, scales, and severe pruritus accompanied by infection or allergies to psychogenic factors, chemicals, or irritants.[1]

The incidence of AD tends to increase 2–3 times in the last 30 years.[1,2] Cohort studies on AD incidence observed that approximately 33%–43% of infants with AD had allergic asthma. Moreover, 38%–45% of AD children develop allergic rhinitis during childhood. A total of 33% of AD patients have been shown to develop both of these diseases before adulthood.[3,4]

AD is a complex skin disease caused by the interaction of various factors such as genetic factors, skin barrier factors, predisposing factors, environmental factors and precipitating factors.[5,6] Precipitating factors vary widely, ranging from irritants, contact and inhaled allergens, and infection with microorganisms. The pathogenesis of AD begins with the entry of allergens captured by APC cells which will then be processed and presented to T cells. After sensitization, T cells will differentiate into Th2 cells, which will trigger the release of IL-4, helping B cells to produce IgE. IgE will occupy receptors on mast cells and basophils, resulting in degranulation and the release of inflammatory mediators (histamine, prostaglandins, cytokines IL-3, IL-4, IL-5). These mediators cause vasodilation, inflammatory reactions (cell migration and adhesion molecules) and itching in skin manifestations. The immune response in AD is similar to a delayed-type response or a type IV reaction because it involves T lymphocytes, but it cannot be classified as an IgE-mediated rapid hypersensitivity, and because AD is IgE-mediated, AD is known as "IgE-mediated delayed-type hypersensitivity".[7,8]

The increase in atopic disease has been explained by two different hypotheses: the "hygiene hypothesis" and the "diet–microbiome hypothesis." The hygiene hypothesis states that an increased incidence of autoimmune and allergic diseases in developed countries is associated with better hygiene and reduced exposure to infections during childhood.[9,10] The diet–microbiome hypothesis provides an alternative explanation that changes in the Western diet (decreased fiber intake and increased fat intake) can alter the composition of the gut microbiome, leading to changes in the production of immunomodulatory molecules, such as a decrease in short-chain fatty acids, which affects hematopoiesis, immune cell function, and the regulation of allergic responses.[8,11] In AD lesions, the proportions of

Staphylococcus aureus and *Staphylococcus epidermidis* appear to be increased, but *Propionibacterium*, *Corynebacterium*, and *Streptococcus* species are reduced during exacerbations. The administration of topical therapy increases the diversity of the microbiome.[12] Thus, this study tried to illustrate bacterial colonization in AD patients.

PATIENTS AND METHODS

A descriptive observational study with a cross-sectional approach was conducted at the Polyclinic of Dermatology and Venereology, General Hospital of the Universitas Sumatera Utara (USU Hospital). The research sample was examined at the Integrated Laboratory of Microbiology of the hospital. This research has been reviewed by the Ethics Committee at Universitas Sumatera Utara No. 653/KEP/USU/2021.

The subjects were children with AD who came to the Polyclinic of Dermatology and Venereology at USU Hospital and were willing to participate in the study by signing an informed consent form. Subjects would be excluded if the parent/guardian cannot answer the question or the child has autoimmune diseases, such as psoriasis and systemic lupus erythematosus. The bacterial culture obtained from 35 pediatric AD patients was examined to determine the description of bacterial colonization.

The samples were taken from both the lesion and non-lesion areas. Every sample from each area will be divided and grown anaerobically and aerobically. The extraction area was cleaned with a cotton swab moistened with 70% alcohol and was allowed to dry. After swabbing the lesion and non-lesion areas with sterile cotton swabs, the extracted bacteria were identified with a Gram stain test. They were inserted into the bacterial growth medium of blood agar and Brucella agar. Cultures were grown under aerobic and anaerobic conditions at 37°C. The bacterial suspension was put into the Vitek® 2 machine, which contains various biochemical tests for bacterial identification, and was examined using the anaerobic card Vitek® 2 and identification card Vitek® 2. The data of bacterial identification in the form of percentages were collected in a descriptive table.

RESULTS

Thirty-five pediatric AD patients at the Polyclinic participated in this study from September 2021 to December 2021. Subjects were dominated by boys, with 18 people (51.4%), and the average age was 7.58 years, with the youngest being 2 months and the oldest being 16 years. Most participants were in the age group of 5–18 years (65.7%). Based on ethnicity, up to 54.3% of participants were Batak, followed by Minang (17.1%), Acehnese (11.4%), Malays (8.6%), Javanese (5.7%), and Indian (2.9%) (Table 1).

Burkholderia cepacia and *Pseudomonas stutzeri* were found on the finger area of a patient. The types of bacteria found on the neck of one patient were *Aerococcus viridans*, *Micrococcus luteus*, *Burkholderia cepacia*, and *Enterobacter cloacae*. In the forearm area, the common bacteria found were *Acinetobacter baumannii* in seven subjects (14.6%), followed by *Staphylococcus hominis* in six subjects (12.5%) and *Burkholderia cepacia* in five subjects (10.4%).

Sphingobacterium thalpophilum and *Staphylococcus sciuri* were observed on a subject's forearm and lower limbs, and another subject had *Kocuria kristinae* and *Sphingomonas paucimobilis* on the forearm and body. *Burkholderia mallei* and *Sphingomonas paucimobilis* were found on the hand folds of one participant. Furthermore, *Kocuria kristinae* was observed on the lower limbs of three subjects (37.5%), and only *Sphingomonas paucimobilis* was identified on the knees of two subjects (Table 2).

The majority of the AD subjects had received treatment (97.1%), and only one person (2.9%) had never received medication (Table 3).

The lesion area of AD patients contained Gram-negative bacteria, namely, *Acinetobacter baumannii* and *Burkholderia cepacia* (14.3%), and Gram-positive bacteria, that is, *Staphylococcus hominis* (15%).

On the non-lesion sites, both Gram-positive and negative bacteria, namely, *Sphingomonas paucimobilis* (14.3%), *Acinetobacter baumannii* (11.4%), *Kocuria kristinae* (11.4%), and *Staphylococcus hominis* (8.6%) (Table 4), were identified.

DISCUSSION

AD is a chronic inflammatory disease most often found in infants and children, with an increased prevalence of up to 30% in recent years worldwide. Its first symptoms are mostly found in the first 12 months of life, and its occurrence decreases with age, with which 45% appear in the first 6 months, 60% in the first 12 months, and 85% before 5 years. Based on the data in Korea, 26.5% of infants under 2 years old were diagnosed with AD, and the rate decreased dramatically at age 3 (11.6%)

Table 1. Demographic Characteristics of Children with AD

Demographic Characteristics	n = 35
Sex, n (%)	
Boy	18 (51.4)
Girl	17 (48.6)
Age, years	
Mean (SD)	7.58 (4.46)
Median (Min–Max)	8 (0.17–16)
<5 years	12 (34.3)
5–18 years	23 (65.7)
Ethnicity, n (%)	
Batak	19 (54.3)
Indian	1 (2.9)
Javanese	2 (5.7)
Malay	3 (8.6)
Minang	6 (17.1)
Acehnese	4 (11.4)

to 19 years (4.6%).[13] Nutten's study found that 50% of AD patients develop allergic symptoms within the first year of life, and 85% of patients have onset under 5 years.[14] AD is more commonly found in children because of their immature immune system, exposure to environmental allergens, and increased awareness of AD.[15] In contrast to the results of this study, more children diagnosed with AD were aged 5–18 years (65.7%) compared to those aged < 5 years (34.3%). Delays in treatment and diagnosis can cause this.

Boys (50.9%) tend to develop AD compared to girls.[16] However, another retrospective study at RSUP by Prof. Dr. R. D. Kandou observed that AD in girls (54.7%) has a higher case than that of boys (45.3%).[16] The cause of the higher predilection between genders is unknown. AD has a longer progression and a lower remission rate in boys than in girls, with early onset during the first year. In infancy, boy AD patients are dominant, but the opposite results were found in adolescence.[13] As in this study, it was found that the prevalence of boys affected by AD was more than that of girls, namely, 51.4% and 48.6%, respectively.

Table 2. Frequency Distribution of Bacterial Characteristics by Rash Location in Children with AD

Rash Location	Bacteria	Freq. (%)
Hand fingers	*Burkholderia cepacia*	1 (50)
	Pseudomonas stutzeri	1 (50)
Neck	*Aerococcus viridans*	1 (25)
	Micrococcus luteus	1 (25)
	Burkholderia cepacia	1 (25)
	Enterobacter cloacae	1 (25)
Forearm	*Acinetobacter baumannii*	7 (14,6)
	Acinetobacter haemolyticus	1 (2,1)
	Acinetobacter lwoffii	1 (2,1)
	Aerococcus urinae	1 (2,1)
	Aeromonas hydrophila	1 (2,1)
	Burkholderia cepacia	5 (10,4)
	Granulicatella adiacens	3 (6,2)
	Kocuria kristinae	1 (2,1)
	Kocuria varians	1 (2,1)
	Micrococcus luteus	2 (4,2)
	Pantoea spp.	3 (6,2)
	Pseudomonas aeruginosa	1 (2,1)
	Pseudomonas stutzeri	1 (2,1)
	Rhizobium radiobacter	2 (4,2)
	Sphingomonas paucimobilis	4 (8,3)
	Staphylococcus aureus	1 (2,1)
	Staphylococcus epidermidis	3 (6,2)
	Staphylococcus hominis	6 (12,5)
	Staphylococcus lentus	3 (6,2)
	Staphylococcus capitis	1 (2,1)
Forearm and lower limb	*Sphingobacterium thalpophilum*	1 (50)
	Staphylococcus sciuri	1 (50)
Forearm and body	*Kocuria kristinae*	1 (50)
	Sphingomonas paucimobilis	1 (50)
Hand fold	*Burkholderia mallei*	1 (50)
	Sphingomonas paucimobilis	1 (50)
Lower limb	*Acinetobacter baumannii*	2 (25)
	Burkholderia cepacian	1 (12,5)
	Kocuria kristinae	3 (37,5)
	Pantoea spp.	1 (12,5)
	Staphylococcus vitulinus	1 (12,5)
Knee	*Sphingomonas paucimobilis*	2 (100)

Many studies suggest that race is associated with AD. Asian races with Asian dermatitis have frequent Th2 activation, and its lesions are well-demarcated with scaling and lichenification.[17] In the United States, children with black skin are more likely to develop AD than white (OR, 1.7). Silverberg also emphasized in 2016 that Black and Asian children are more likely to experience AD.[18] Kim et al. found that the incidence and persistence of AD were higher in nonwhite skin races.[19] Research in Indonesia regarding racial differences and the incidence of AD was not found until this study was written.

The rash caused by AD is common at the fold sites. It can also be found on the anterior and lateral neck, forehead, face, wrists, and backs of the feet and hands.[20] Local skin physiology can determine the role of the microbiota in AD prognosis. Several studies analyzed the effect of different skin locations and physiological components, such as the fat profile on microbiome levels, on AD. The epidermal fat composition was significantly related to bacterial diversity and composition at AD predilection sites. Higher levels of long-chain unsaturated fatty acids were found to be associated with an increase in lipophilic *Propionibacteria* and *Corynebacteria*.[21,22]

The manifestation site of AD is diverse based on age. Cheek sites are most frequent in infants, and joint extensor and joint flexures are more common in children and older children. AD causes immune dysregulation that leads to IgE production. Bacteria culture examination showed the presence of dysbiosis, in which Staphylococcus aureus colonies caused 70% of lesions and 39% of non-lesions on the skin. *Staphylococcus aureus* predominated at the affected skin compared to control, particularly at the site of inflammation (compared to xerosis) and during flares in untreated patients. Other microbiomes were found on the affected skins, such as Staphylococcus epidermidis and Staphylococcus haemolyticus.[23]

It is caused by mutations in the gene encoding the filament aggregating protein (filaggrin), which causes the absence of predisposing functional proteins

as a barrier and disruption of the skin microbiome, resulting in AD, increased stratum corneum pH, and increased susceptibility to recurrent bacterial skin infections among patients with AD.[23,24]

The anatomical structure, such as sweat glands, hair follicles, and sebaceous glands influence the skin microbiome. The antecubital and popliteal fossa are the typical sites for AD, with the predominance of the phyla Actinobacteria (genus *Propionibacterium* and *Corynebacterium*), Firmicutes (genus *Staphylococcus*), Proteobacteria, and Bacteroidetes. *Staphylococcus epidermidis* is often commensal and colonizes the head, axilla, and nasal region.[24]

AD skin has a lower bacterial diversity than control skin, which is reduced during flares. It was noted that a decrease in species from the *Streptococcus*, *Propionibacterium*, *Acinetobacter*, *Corynebacterium*, and *Prevotella* genres was associated with an increase in *S. aureus*. One study found that infants diagnosed with AD at 2 months old had lower commensal *Staphylococcus* spp. levels on the antecubital fossa at 12 months than on the unaffected skin at the same age.[23]

This study observed intermittent therapy decreased *S. aureus* dominance and obliterated diversity during flares. However, another study found that therapy does not affect bacterial diversity. Bjerre et al. used a moisturizer for 28 days and recorded no changes in genus-level microflora at certain skin sites, but *S. aureus* increased in the nonmoisturizing control group.[23] Following successful topical AD treatment, there was an increase in skin microbiome diversity emerging from existing taxa in the observed skin microbiome.[12]

The microbiome triggers the innate immune response in the skin to attack microbes through the production of antimicrobial peptides, namely, cathelicidins and β-defensins. Skin commensals then modulate immune system development through the promotion of type 1 T-helper via Interleukin 1 signaling by inhibiting Th2, which is responsible for allergic conditions.[12,24]

Among the Gram-positive *Staphylococcus* species, *Staphylococcus epidermidis* is the major type on healthy skin, capable of inhibiting the growth of *Staphylococcus aureus*. In children, skin colonization by *Staphylococcus epidermidis* and *Staphylococcus cohnii* during the first year of life has a protective effect on AD progression. The increased proportions of *Staphylococcus aureus* and *Staphylococcus epidermidis* in AD are due to the production of antibacterial compounds (antimicrobial peptides and bacteriocins), which causes a relative decrease during flares in other species of *Propionibacterium*, *Corynebacterium*, and *Streptococcus*.[12]

Studies on AD microbiota showed that flares are associated with microbial dysbiosis, which is dominated by *Staphylococcus aureus* up to 90%.[25] *Staphylococcus aureus* and *Streptococcus*

Table 3. Frequency Distribution of Bacterial Characteristics Based on Medical History in Children with AD

History	Freq. (%)	Lesion	Gram	Bacteria
Yes	34 (97.1)	Lesion	Gram-Negative Bacilli	*Acinetobacter baumannii*
				Acinetobacter haemolyticus
				Aeromonas hydrophila
				Burkholderia cepacia
				Burkholderia mallei
				Pantoea spp.
				Pseudomonas aeruginosa
				Pseudomonas stutzeri
				Rhizobium radiobacter
				Sphingobacterium thalpophilum
				Sphingomonas paucimobilis
			Gram-Positive Coccus	*Aerococcus urinae*
				Granulicatella adiacens
				Kocuria kristinae
				Staphylococcus aureus
				Staphylococcus epidermidis
				Staphylococcus hominis
				Staphylococcus vitulinus
		Nonlesion	Gram-Negative Bacilli	*Acinetobacter baumannii*
				Acinetobacter lwoffii
				Burkholderia cepacian
				Enterobacter cloacae
				Pantoea spp.
				Pseudomonas stutzeri
				Rhizobium radiobacter
				Sphingomonas paucimobilis
			Gram-Positive Coccus	*Granulicatella adiacens*
				Kocuria kristinae
				Kocuria varians
				Micrococcus luteus
				Staphylococcus epidermidis
				Staphylococcus hominis
				Staphylococcus lentus
				Staphylococcus sciuri
				Staphylococcus capitis
No	1 (2.9)	Lesion	Gram-Negative Bacilli	-
			Gram-Positive Coccus	*Aerococcus viridans*
		Non-lesion	Gram-Negative Bacilli	-
			Gram-Positive Coccus	*Staphylococcus hominis*

Table 4. Bacterial Colonization Profile in Pediatric AD Patients

Morphology	Gram	Bacteria	Freq. (%)
Lesion	Gram-Negative Bacilli	*Acinetobacter baumannii*	5 (14.3)
		Acinetobacter haemolyticus	1 (2.9)
		Aeromonas hydrophila	1 (2.9)
		Burkholderia cepacia	5 (14.3)
		Burkholderia mallei	1 (2.9)
		Pantoea spp.	2 (5.7)
		Pseudomonas aeruginosa	1 (2.9)
		Pseudomonas stutzeri	1 (2.9)
		Rhizobium radiobacter	1 (2.9)
		Sphingobacterium thalpophilum	1 (2.9)
		Sphingomonas paucimobilis	3 (8.6)
	Gram-Positive Coccus	*Aerococcus urinae*	1 (2.9)
		Aerococcus viridans	1 (2.9)
		Granulicatella adiacens	1 (2.9)
		Kocuria kristinae	1 (2.9)
		Staphylococcus aureus	1 (2.9)
		Staphylococcus epidermidis	2 (5.7)
		Staphylococcus hominis	4 (11.4)
		Staphylococcus vitulinus	1 (2.9)
		Total	**35 (100)**
Non-lesion	Gram-Negative Bacilli	*Acinetobacter baumannii*	4 (11.4)
		Acinetobacter lwoffii	1 (2.9)
		Burkholderia cepacian	3 (8.6)
		Enterobacter cloacae	1 (2.9)
		Pantoea spp.	2 (5.7)
		Pseudomonas stutzeri	1 (2.9)
		Rhizobium radiobacter	1 (2.9)
		Sphingomonas paucimobilis	5 (14.3)
	Gram-Positive Coccus	*Granulicatella adiacens*	2 (5.7)
		Kocuria kristinae	4 (11.4)
		Kocuria varians	1 (2.9)
		Micrococcus luteus	2 (5.7)
		Staphylococcus epidermidis	1 (2.9)
		Staphylococcus hominis	3 (8.6)
		Staphylococcus lentus	2 (5.7)
		Staphylococcus sciuri	1 (2.9)
		Staphylococcus capitis	1 (2.9)
		Total	**35 (100)**

spp. are discovered in 70%–80% of AD patients with infected lesions. *Staphylococcus aureus* was found in 70% of skin lesions, 39% of non-lesion skin, and 62% on the nose. A meta-analysis study showed predominant colonization of skin lesions (OR, 19.74; 95% CI, 10.88–35.81; $p < 0.001$), non-lesion skin (OR, 7.77; 95% CI, 3.82–15.82; $p < 0.001$), and nose (OR, 4.50; 95% CI, 3.00–6.75; $p < 0.001$).[26]

Bilal et al. in a study in Saudi Arabia uncovered that the presence of Streptococcus infection and colonization of Gram-negative bacteria exacerbated AD in children. A total of 240 bacterial colonies from AD lesions were compared with 193 colonies from non-lesion skin. Gram-positive cocci were found in 78 (97.5%) lesions and in 77 (96.2%) non-lesion skin. *Staphylococcus* species were significantly detected in the lesion skin compared to the non-lesion one. *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, and *Corynebacterium minutissum* were significantly isolated from the lesions compared with non-lesion skin, whereas *Corynebacterium xerosis* was more abundant in the lesions ($p = 0.21$). Gram-negative bacteria were isolated from seven (8.8%) lesions but none from non-lesion skin. The species identified were *Pantoea agglomerans*, *Enterobacter cloacae*, *Chryseobacterium indologenes*, and *Acinetobacter lwoffii*.[27]

CONCLUSION

Differences were found in examining bacterial cultures carried out in lesional and non-lesional AD patients. There were 4 types of bacteria found on the neck, 20 types on the forearm, 2 types on hand folds, 2 types on fingers, 1 type on the knee, and 5 types on lower limbs. The frequently found bacteria were Gram-negative bacilli, in which *Acinetobacter baumannii* (15%) and *Burkholderia cepacian* (14.3%) were dominant in five patients, and Gram-positive coccus, *Staphylococcus hominis*, was found in four patients (11.4%). Meanwhile, the most gram-negative bacilli found in non-lesion were *Sphingomonas paucimobilis* in 5 people (14.3%), and the most gram-positive coccus bacteria was *Kocuria kristinae* in 4 people (11.4%).

DISCLOSURES

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Conflict of Interest

None of the author have any conflicts of interest to disclose.

Ethical Statement

This research has been reviewed by the Ethics Committee at Universitas Sumatera Utara No. 653/KEP/USU/2021.

Author Contributions

All authors involved in concepting designing and supervising the manuscripts and Deryne Anggia Paramita conducted the research. All authors analyzed the data. All authors prepare the manuscript and agree for this final version of manuscript to be submitted to this journal.

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