Prevalence of Cutaneous Bacterial Colonization in Thai Patients with Psoriasis

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Objective: To determine bacterial colonization of skin, scalp, nares, nails, and psoriasis lesion in Thai psoriasis patients and compare findings with those of healthy controls.

Material and Method: Sixty patients with chronic plaque-type psoriasis and 60 healthy controls of similar age and gender were enrolled. Swabs of nares, scalp, nails, and non-lesional skin were taken from all subjects. Swabs of lesional skin were taken from psoriasis patients. Aerobic bacteria were isolated from swab specimens.

Results: Patients with psoriasis had significantly higher rate of bacteria colonization in nares, scalp, and nails than those of healthy controls. Firmicutes spp. was the most common phyla, followed by Proteobacteria spp. in both groups. Coagulase-negative staphylococci (CoNS) were the most common pathogens isolated from lesional skin, non-lesional skin, scalp, and nares of psoriasis patients. Streptococcus spp. was found only in psoriasis patients.

Conclusion: Similar to findings from Caucasian psoriasis patients, Firmicutes spp. was found to be the most common phyla colonizing the skin of Asian psoriasis patients. Streptococcus spp. was found to colonize only the skin. Further studies are needed to determine the clinical significance of streptococcal skin colonization in psoriasis patients.

Keywords: Bacteria, Colonization, Psoriasis

After birth, environmental microbes rapidly begin to colonize the stratum corneum, which eventually develops into a microbial ecosystem[1]. Human skin microbiota includes all microbial members found in a particular cutaneous site habitat[2]. Resident microbiota refers to normal commensal skin microbiota that is in homeostasis with its host. Transient microbiota refers to environmental microbes that live on the skin temporarily. The microbiome of human skin is highly diverse and has a high degree of interpersonal variation according to various intrinsic and extrinsic factors. Genotype, age, gender, and topographical regions of the body having different characteristics (pH, moisture, salinity, and sebum content) are the most important intrinsic factors. Extrinsic individual factors include occupation, lifestyle, geographic location, and use of antibiotics and/or cosmetics[3].

Psoriasis is a chronic inflammatory disease of the skin that is characterized by well-defined scaly erythematous plaque. *Staphylococcus aureus* (*S. aureus*) and its toxigenic strains and increased numbers of group A streptococcal-specific T helper 1 (Th1) cells have been found to associate with psoriasis[4,5]. Previous studies using molecular techniques targeting 16S rRNA genes to investigate bacterial flora from skin swabs and skin biopsies revealed that *Firmicutes* spp., *Proteobacteria* spp., and *Actinobacteria* spp. were the three most common phyla in the skin of both healthy patients and psoriasis patients[6,7]. Most of the studies were conducted in Caucasians in the United States, Sweden, and Austria[5,7,8]. There is, however, little data regarding bacterial colonization of psoriasis skin in Asian populations[4].

As mentioned previously, genotype, lifestyle, and geographic location affects human microbiota. The aim of the present study was to determine bacterial colonization of skin, scalp, nares, nails, and psoriasis lesion in Thai psoriasis patients and compare findings with those of healthy controls.

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Material and Method
Patients and matching healthy controls

Sixty patients older than 18 years with chronic plaque-type psoriasis and 60 healthy controls with same gender ratio, ethnicity, and similar age (±2 years) to each psoriasis patient were enrolled between August 2014 and November 2014. None of the psoriasis patients had been exposed to systemic immunosuppressive drugs, apart from methotrexate and cyclosporine, for one month prior to enrollment in the study. We excluded psoriasis patients and healthy controls who: (i) had been exposed to systemic and/or topical antibiotics and/or intranasal steroids within one month of enrollment, (ii) had clinical signs of cutaneous and/or systemic infection, and/or (iii) had been admitted to hospitals within two months of enrollment. All patients and healthy controls were recruited from the same geographic region (Bangkok, Thailand). For each psoriasis patient, onset of disease, disease duration, previous treatment (three months prior to enrollment), current treatment, and Psoriasis Area and Severity Index (PASI) were recorded.

Specimen collection and processing

In patients with psoriasis, skin swabs were taken from nares, scalp, lesional skin, and non-lesional skin beyond 5 centimeters from the swabbed lesion. Specimens of lesional skin were collected for culture in the following order: trunk, arm, and leg. In healthy controls, skin swabs were taken from nares, scalp, and normal skin; at all times attempting to match as closely as possible the body site affected in psoriasis patients. The skin area to be swabbed was wiped with sterile gauze and normal saline to remove contaminating bacteria and ointment residues. A sterile cotton swab was then dipped in sterile distilled water and rubbed repeatedly on a 1 cm^2 targeted area of the skin for five seconds using sufficient pressure[19]. This swabbing process was repeated for each area of the body in both psoriasis patients and healthy controls. Smear material was applied on sheep blood agar plates and MacConkey agar plates. To obtain nail specimens from both psoriasis patients and healthy controls, a nail curette was used to scrape the underside of the nail plate, with specimen material being sent to the laboratory for bacteria culture. Culture samples were frozen in Microbank™ storage boxes (Pro-Lab Diagnostics, Westwood Court, United Kingdom) according to manufacturer's instructions. Inoculated plates were incubated aerobically at 35°C for 24 hours. Grown isolates were then identified by biochemical tests.

Processing and culture of collected specimens were performed at the laboratory of the Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University.

PASW Statistics version 18 (SPSS, Inc., Chicago, IL, USA) was used to perform statistical analysis. Descriptive statistics (number and percentage; mean ± standard deviation) were used to describe demographic data. Association between two categorical variables was compared using Chi-square test. The p-value of less than 0.05 were considered to be statistically significant. This study was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Results

Sixty patients with psoriasis (28 men and 32 women, mean age 46.0±14.6 years) and 60 healthy controls (28 men and 32 women, mean age 45.5±14.6 years) were enrolled. Mean age at onset and disease duration in patients with psoriasis were 31.8±14.9 years and 14.2±11.8 years, respectively. At the beginning of the study, 27 patients used only topical treatment, whereas, 33 patients received phototherapy and systemic treatment, such as methotrexate, acitretin, and/or cyclosporine. Mean PASI score of psoriasis patients was 6.0±5.5. In both psoriasis and healthy control groups, skin swabs were taken from trunk, arms, and legs in 76.7%, 18.3%, and 5%, respectively. Bacterial colonization in different parts of the body in psoriasis patients and healthy controls is described in Table 1. Patients with psoriasis had significantly higher detection rate of bacterial colonization in nares, scalp, and nails than those of healthy controls. The most common phyla in psoriasis plaque and normal skin of psoriasis patients and skin of healthy controls were *Firmicutes* spp., followed by *Proteobacteria* spp. (Table 2). Similar phyla-related findings were also observed in nares, scalp, and nails of psoriasis patients and healthy controls. Coagulase-negative staphylococci (CoNS) were the most common bacteria found in lesional skin, normal skin, and nares of patients with psoriasis.

Streptococcus spp. colonization was found in 3.3%, 1.7%, 8.3%, and 1.7% of involved skin, uninvolved skin, nares, and scalp in psoriasis patients, respectively. No *Streptococcus* spp. colonization was found in healthy controls. Psoriasis patients had greater richness of diversity of cutaneous microbiota than

Table 1. Bacterial colonization of different regions of the body in psoriasis patients and healthy controls

Sites	Psoriasis (n = 60)	Healthy controls (n = 60)	p-value	Odds ratio (95% CI)
Scalp, n (%)	22 (36.7)	9 (15.0)	0.007*	3.3 (1.4-8.0)
Nares, n (%)	52 (86.7)	32 (53.3)	<0.001*	5.7 (1.4-2.3)
Normal skin, n (%)	22 (36.7)	15 (25.0)	0.166	1.3 (0.9-2.1)
Nail, n (%)	38 (63.3)	26 (43.3)	0.028*	2.3 (1.1-4.7)
Psoriasis lesion, n (%)	19 (35.0)	-	-	-

Table 2. Bacterial colonization of different regions of the body in psoriasis patients relative to topical and systemic treatment

	Psoriasis patients (n = 60)		p-value	Odds ratio (95% CI)
	Topical therapy (n = 27) positive culture, n (%)	Systemic therapy (n = 33) positive culture, n (%)		
Scalp	10 (37.4)	12 (36.4)	0.96	0.97 (0.34-2.79)
Nares	22 (81.5)	30 (90.9)	0.45	2.27 (0.49-10.53)
Normal skin	8 (29.6)	14 (42.4)	0.31	1.75 (0.60-5.14)
Nail	14 (51.9)	24 (72.7)	0.10	2.48 (0.85-7.26)
Psoriasis lesion	8 (29.6)	11 (33.3)	0.76	1.19 (0.40-3.56)

healthy controls (Table 3). The most common bacterial colonization of the nails in patients with psoriasis was *Bacillus* spp., which was significantly different from healthy controls (p-value = 0.028; odds ratio: 2.3, 95% CI: 1.1-4.7). Disease severity (PASI score) and type of treatment (topical or systemic treatment) were found not to be significantly associated with presence or absence of cutaneous microbiota (data were not shown).

Discussion

Actinobacteria spp., *Proteobacteria* spp., *Firmicutes* spp., and *Bacteroides* spp. were found to be the four dominant phyla of bacterial microbiota in normal human skin[9]. A compromised skin barrier resulting from higher basal level of transepidermal water loss and/or decreased hydration of the stratum corneum may render psoriasis skin an unsuitable habitat for certain organisms that survive in normal skin[10]. However, two studies from the United States and one study from Sweden that used molecular technique to investigate bacterial flora from skin swabs and skin biopsies of psoriasis patients showed that these four phyla were also predominate in psoriasis plaques[7,8,11]. Studies by Gao et al[11] (USA, skin swabs), Fahlén et al[7] (Sweden, skin biopsies), and the present study (Thailand, skin swabs) showed that *Firmicutes* spp. was the most common phyla of bacterial colonization in psoriasis patients. The study by Alekseyenko et al (USA, skin swabs) demonstrated that *Firmicutes* spp. and *Actinobacteria* spp. were

significantly enriched in psoriasis lesions when compared to healthy controls[8]. These findings may suggest a strong association between *Firmicutes* phyla and lesional skin of psoriasis patients, even in different genotypes and geographical regions.

In 1976, Aly et al[12] (USA) used skin swab and culture technique to identify aerobic bacterial flora in involved skin, adjacent non-involved skin, and anterior nares of 40 psoriasis patients. They reported that coagulase negative staphylococci (CoNS) were the predominate organism in all three areas of psoriasis patients, which is similar to the finding from our study[12]. *S. aureus* and *Streptococcus* spp. have been proposed to be superantigens of psoriasis and enterotoxins of *S. aureus* may be associated with severity of disease[4,5,13]. Although several studies reported carriage rate of *S. aureus* in nares and lesional skin of psoriasis patients to be higher than in that of healthy controls, our study and the study by Aly et al did not show similar results[4,5,12-15]. In our study, streptococcal colonization was found in psoriasis patients, but not in healthy controls. Although streptococcal sore throat is known to be one of the trigger factors that can aggravate guttate and chronic plaque-type psoriasis, only a few cases of streptococcal skin infection-induced psoriasis have been reported[16]. Further studies are needed to determine if any significant associations exist between streptococcal colonization of the skin and psoriasis.

The major limitation of our study was that we used aerobic culture technique to investigate microbial

Table 3. Types of bacteria found to colonize different regions of the body in psoriasis patients and healthy controls

Site	Micro-organisms	Psoriasis (n = 60)	Healthy controls (n = 60)	p-value	Odds ratio[+] (95% CI)
Scalp	Firmicutes (phyla)	18 (30.0%)	8 (13.3%)	0.027*	2.80 (1.0-7.8)
	- Coagulase-negative staphylococci	15 (25.0%)	8 (13.3%)		
	- *Staphylococcus aureus*	-	-		
	- *Streptococcus* spp.	1 (1.7%)	-		
	- *Bacillus* spp.	2 (3.3%)	-		
	Proteobacteria (phyla)	2 (3.3%)	1 (1.7%)	0.55	2.00 (0.2-23.1)
	- *Acinetobacter baumannii*	-	1 (1.7%)		
	- *Enterobacter* spp.	1 (1.7%)	-		
	- *Eschericia coli*	-	-		
	- *Klebsiella pneumonia*	1 (1.7%)	-		
	- *Klebsiella* spp.	-	-		
Nares	Firmicutes (phyla)	49 (81.7%)	26 (43.3%)	<0.001*	4.10 (1.6-10.7)
	- Coagulase-negative staphylococci	33 (55.0%)	21 (35.0%)		
	- *Staphylococcus aureus*	7 (11.7%)	5 (8.3%)		
	- *Streptococcus* spp.	5 (8.3%)	-		
	- *Bacillus* spp.	4 (6.7%)	-		
	Proteobacteria (phyla)	7 (11.7%)	6 (10.0%)	0.768	1.20 (0.4-3.8)
	- *Acinetobacter baumannii*	2 (3.3%)	-		
	- *Enterobacter* spp.	3 (5.0%)	-		
	- *Eschericia coli*	1 (1.7%)	1 (1.7%)		
	- *Klebsiella pneumonia*	1 (1.7%)	3 (5.0%)		
	- *Klebsiella* spp.	-	1 (1.7%)		
	- Non-fermenting bacteria	-	1 (1.7%)		
Normal skin	Firmicutes (phyla)	21 (35.0%)	15 (25.0%)	0.23	1.62 (0.7-3.6)
	- Coagulase-negative staphylococci	12 (20.0%)	14 (23.3%)		
	- *Staphylococcus aureus*	-	1 (1.7%)		
	- *Streptococcus* spp.	1 (1.7%)	-		
	- *Bacillus* spp.	8 (13.3%)	-		
	Proteobacteria (phyla)	1 (1.7%)	-	-	-
	- *Acinetobacter baumannii*	-	-		
	- *Enterobacter* spp.	1 (1.7%)	-		
Nail	Firmicutes (phyla)	38 (63.3%)	26 (43.3%)	0.028*	2.26 (1.0-5.0)
	- Coagulase-negative staphylococci	14 (23.3%)	17 (28.3%)		
	- *Staphylococcus aureus*	-	1 (1.7%)		
	- *Streptococcus* spp.	-	-		
	- *Bacillus* spp.	24 (40.0%)	8 (13.3%)		
	Proteobacteria (phyla)**	-	-	-	-
Psoriasis lesion	Firmicutes (phyla)	20 (33.3%)	-	-	-
	- Coagulase-negative staphylococci	11 (18.3%)	-		
	- *Staphylococcus aureus*	2 (3.3%)	-		
	- *Streptococcus* spp.	2 (3.3%)	-		
	- *Bacillus* spp.	5 (8.3%)	-		
	Proteobacteria (phyla)	1 (1.7%)	-	-	-
	- *Acinetobacter baumannii*	1 (1.7%)	-		

[+] Odds ratio (95% CI) compared positive culture to phyla between psoriasis and healthy controls
** No Proteobacteria (phyla) colonization was found in nails of psoriasis and healthy controls

communities. This method may underestimate bacterial skin colonization, as the percentage of bacteria that are able to thrive in standard laboratory conditions tends to be less than 10%[18]. Molecular-based techniques, like 16S rRNA gene, provide greater accuracy and less bias. A large study by Alekseyenko et al (USA) of 51 psoriasis patients that used a molecular-based approach showed that psoriasis plaque contained an

increased, combined relative abundance of *Streptococcus* spp., *Staphylococcus* spp., *Corynebacterium* spp., and *Propionibacterium* spp. However, only the combination of *Streptococcus* spp., *Staphylococcus* spp., and *Corynebacterium* spp. in psoriasis plaque was significantly different from uninvolved skin and controls[8,18]. Thus, *Propionibacterium* spp. (anaerobic bacteria), which was not able to be cultured in our study, may not have had a significant effect on our results. Based on our review of the literature, there is little published data and evidence regarding skin microbiota of psoriasis patients in tropical Asian countries. Our study may provide foundational data regarding skin microbiota in Asian patients with psoriasis.

In conclusion, our study showed that *Firmicutes* spp. was the most common phyla in psoriasis plaque in a tropical Asian country. CoNS were the most common pathogens detected in involved skin, uninvolved skin, and nares of psoriasis patients. *Streptococcus* spp. was detected in the skin of only psoriasis patients in our study. Further studies are needed to determine clinical significance of *Streptococcus* colonization in Asian patients with psoriasis.

What is already known on this topic?

Firmicutes spp. was found to be the predominant phyla colonizing the skin in Caucasian patients with psoriasis.

What this study adds?

Firmicutes spp. was found to be the most common phyla in psoriasis plaque in both Caucasian and Asian populations, even in different genotypes and geographical regions.

Potential conflicts of interest

None.

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ความชุกของเชื้อแบคทีเรียที่ผิวหนังของผู้ป่วยไทยโรคสะเก็ดเงิน

ลีนา จุฬาโรจน์มนตรี, ชนิษฎา วงษ์ประภารัตน์, ปภาพิต ตู้จินดา, วรนรี วินะยานุวัติคุณ, อธิรัฐ บุญญาศิริ, กนกวลัย กุลทนันทน์, วิษณุ ธรรมลิขิตกุล

วัตถุประสงค์: เพื่อเปรียบเทียบความชุกของแบคทีเรียที่ผิวหนัง หนังศีรษะ โพรงจมูก และเล็บของคนไทยที่เป็นและไม่ได้เป็นโรคสะเก็ดเงิน

วัสดุและวิธีการ: ผู้ป่วยโรคสะเก็ดเงินชนิดปื้นหนา 60 ราย และอาสาสมัครปกติ 60 ราย ที่มีอายุใกล้เคียงกันและเพศเดียวกันได้รับการเก็บตัวอย่างจากผิวหนังปกติ หนังศีรษะ โพรงจมูกทั้งสองข้าง เล็บ เพื่อไปเพาะหาเชื้อแบคทีเรีย กลุ่มผู้ป่วยได้เก็บตัวอย่างที่ผื่นสะเก็ดเงินด้วย

ผลการศึกษา: ความชุกของแบคทีเรียที่บริเวณโพรงจมูก หนังศีรษะ และเล็บในกลุ่มผู้ป่วยมากกว่ากลุ่มอาสาสมัครปกติอย่างมีนัยสำคัญทางสถิติ phyla ของเชื้อที่พบมากที่สุด คือ Firmicutes spp. รองลงมาคือ Proteobacteria spp. ในผู้ป่วยทั้งสองกลุ่มผู้ป่วยโรคสะเก็ดเงินพบเชื้อ coagulase-negative staphylococci มากที่สุดในทุกตำแหน่งยกเว้นเล็บ ส่วนเชื้อ Streptococcus spp. พบเฉพาะในผู้ป่วยโรคสะเก็ดเงิน

สรุป: เชื้อกลุ่ม Firmicutes spp. เป็นเชื้อที่พบมากที่สุดในผู้ป่วยโรคสะเก็ดเงินชาวเอเชียน ส่วนเชื้อ Streptococcus spp. ซึ่งพบเฉพาะในผู้ป่วยโรคสะเก็ดเงินนั้นต้องศึกษาเพิ่มเติมเพื่อหาความสำคัญทางคลินิกต่อไป