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Acidity difference and skin *Staphylococcus* spp. colonization in preterm and term newborns

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Abstract

Introduction: Term and preterm newborns have differences in skin structure. Preterm newborns have skin structures that are more susceptible to various disorders. These differences are believed to impact skin pH regulation and bacterial colonization, particularly *Staphylococcus* spp. This study investigates the acidity difference and skin *Staphylococcus* spp. colonization in preterm and term newborns.
Methods: This is a descriptive observational study with a cross-sectional design. The study compared skin pH and *Staphylococcus* spp. colonization between term (37–41 weeks) and preterm (28–36 weeks) newborns. Skin pH was measured using a skin pH meter, and *Staphylococcus* spp. colonization was assessed through bacterial identification and colony-forming unit (CFU) testing. Assessments were conducted within the first 24 hours of life.
Results: There were 29 participants in this study: 16 preterm newborns and 13 term newborns. The preterm group was dominated by subjects with alkaline skin pH (80%), and the term group was dominated by subjects with acidic skin pH (71%). Positive *Staphylococcus* spp. colonization was more prevalent in the preterm group (62%) than in the term group (38%).
Conclusions: Skin pH differed between preterm and term newborns, with relatively higher skin pH in the preterm group. *Staphylococcus* spp. was also more prevalent in the preterm group.

Keywords: newborn, term, preterm, skin pH, *Staphylococcus* spp.

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Introduction

The development of neonatal skin begins in utero and continues until approximately 2 years of age (1). Compared to adult skin, neonatal skin exhibits distinct characteristics, including a thinner epidermis and stratum corneum, as well as higher hydration levels (2). Differences in skin condition are also evident between preterm and term newborns. The stratum corneum of preterm newborns consists of only two to three layers, whereas term newborns have 10 to 20 layers, providing greater structural integrity (3). Consequently, preterm newborns are more vulnerable to thermal, mechanical, chemical, and infectious insults (4). Moreover, at birth, newborns undergo a rapid environmental transition from the warm, sterile, fluid-rich intrauterine environment to the cold, dry, and microbe-rich external environment (5).

The stratum corneum plays a crucial role in regulating skin pH (6). Age is one of the most important determinants of skin pH (7). Within the first 3 days of life, skin pH decreases to below 5, establishing the so-called "acid mantle." This acidic environment promotes stratum corneum maturation, homeostasis, and antimicrobial defense (3). Regulation of skin pH occurs through mechanisms such as sodium–hydrogen exchanger 1 (NHE-1) activity, which increases H^+ concentration, thereby maintaining an acidic environment that suppresses pathogenic colonization (8). Commensal microorganisms such as *Lactobacillus* thrive under acidic conditions, whereas potential pathogens including *Staphylococcus* spp., *Cutibacterium*, and *Corynebacterium* proliferate more readily in alkaline environments (9). Acidic pH is also required for the activity of key epidermal enzymes such as β-glucocerebrosidase and acid sphingomyelinase, which regulate ceramide levels and contribute to stratum corneum barrier function (10, 11).

Staphylococcus spp. are Gram-positive cocci commonly found as part of the normal skin flora (12). Under conditions of impaired barrier function, however, these organisms may contribute to various skin disorders (13). Among them, *S. aureus* and *S. epidermidis* are the most frequently encountered species (14). Dysbiosis involving *Staphylococcus* spp. has been linked to skin pH alterations (15, 16). Colonization is favored under more alkaline pH conditions, which facilitate *Staphylococcus* overgrowth and disruption of commensal microbial communities. For instance, *S. aureus* grows optimally at near-neutral to slightly alkaline pH (17). Variations in pH influence bacterial proliferation by affecting protein integrity, enzymatic function, and energy availability (18). Therefore, this study investigates the acidity difference and skin *Staphylococcus* spp. colonization in preterm and term newborns. The findings are expected to provide additional evidence for the role of skin pH in neonatal microbial colonization and may contribute to improved skin care strategies that support optimal barrier function in newborns.

Methods

This was a descriptive observational analytic study with a cross-sectional design. The study was conducted in the neonatal ward and microbiology laboratory of Dr. Moewardi General Hospital,

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Surakarta, Indonesia, between March and April 2025. The study population consisted of newborns born at the hospital during this period, including both preterm and term newborns. Inclusion criteria were gestational age at delivery between 28 and 41 weeks determined based on the last menstrual period or ultrasound examination recorded in the medical report, age less than 24 hours at the time of enrollment, stable vital signs, a 5-minute APGAR score ≥ 7, and delivery by cesarean section, for either absolute or relative indications. Exclusion criteria included fetal distress, intrauterine infection, and major congenital anomalies. The study protocol was reviewed and approved by the Health Research Ethics Committee of Dr. Moewardi General Hospital, Surakarta. Written informed consent was obtained from the parents of all participants.

Two main parameters were assessed: skin pH and *Staphylococcus* spp. colonization. Prior to measurement, each newborn underwent a 20-minute acclimatization period at a room temperature 20 to 22 °C. During acclimatization, skin swab samples were collected from the axilla for microbiological analysis. Following acclimatization, skin pH was measured on the volar forearm and axilla.

Skin pH was measured using a calibrated skin pH meter (Horiba electrode 6261-10C). The newborn was positioned as close as possible to a neutral anatomical position. The measurement area was gently cleaned with a dry tissue without additives. The electrode surface was moistened with distilled water before placement on the skin. The electrode was held perpendicular to the skin surface with minimal pressure to ensure optimal contact without altering skin hydration. Readings were recorded once stabilized, and each site was measured three times. The mean of three measurements was used for analysis.

For *Staphylococcus* spp. colonization, samples were obtained using sterile cotton swabs pre-moistened with 0.9% NaCl. Swabs were collected from the volar forearm and axilla and immediately transferred into transport agar tubes labeled with patient identifiers and the sampling date. Samples were cultured on mannitol salt agar and incubated at 37 °C for 48 hours. Colony counts were performed, and bacterial identification was confirmed using the VITEK® 2 automated system (bioMérieux, Marcy-l'Étoile, France).

Results

A total of 29 newborns met the inclusion and exclusion criteria and were enrolled between March and April 2025. Table 1 summarizes the baseline characteristics of participants. The average age of the mothers was 30.72 years. Most of the research subjects had a gestational age of less than term (55.2%), whereas 44.8% had a term gestational age. The proportion of female subjects (58.6%) was higher than that of male subjects (41.4%). Most of the subjects (55.2%) were in the average birth-weight category (≥ 2,500 g), and the rest (44.8%) were in the low birth-weight (< 2,500 g) category. The body temperature of most subjects was 36 to 36.4 °C (58.6%), and some had a body temperature of 36.5 to 37 °C (41.4%). Room temperature was mostly 25.8 to 36 °C (51.7%) and some had a room temperature of 22 to 25.7 °C (48.3%). The average room humidity was 63.4% with a median value of 63.0%, a minimum value of 50%, and a maximum value of 76%, with most subjects having a humidity of 62% to 76% (55.2%). Most subjects were not placed in an incubator (69.0%); only nine subjects were placed in an incubator (31.0%). A positive maternal atopy history was found in 41.4% of cases, which was lower than the negative maternal atopy history (58.6%). All mothers in this study received antibiotic prophylaxis before undergoing cesarean section, with cefazoline being the most commonly used antibiotic (51.7%)

The distribution of bacterial species differed between groups (Table 2, Fig. 1). *S. hominis* was the most frequently isolated species in preterm newborns, whereas *S. haemolyticus* predominated among term newborns.

Preterm newborns were more likely to have alkaline skin pH (80.0%), whereas term newborns predominantly exhibited acidic skin pH. Meanwhile, in *Staphylococcus* spp. colonization, 61.9% of preterm newborns had positive swab results compared to 37.5% of term newborns (Table 3, Fig. 2).

As shown in Table 4, newborns with alkaline skin pH and positive swab results exhibited a higher mean colony count of *Staphylococcus* spp. compared to those with acidic skin pH and positive swabs.

Table 1 | **Baseline characteristics.**

Characteristics	*n*	%
Maternal age	30.7 ± 5.0	31.0 (20–40)
Gestational age		
Preterm	16	55.2
Term	13	44.8
Sex		
Male	12	41.4
Female	17	58.6
Birthweight		
Low (965–2,490 g)	13	44.8
Normal (2,500–3,910 g)	16	55.2
Body temperature		
36–36.4 °C	17	58.6
36.5–37 °C	12	41.4
Room temperature	26.0 ± 2.5	25.8 (22–36)
25.8–36 °C	15	51.7
22–25.7 °C	14	48.3
Room humidity	63.4 ± 6.2	62.0 (50–76)
62%–76%	16	55.2
50%–61%	13	44.8
Incubator use		
Yes	9	31.0
No	20	60.0
Maternal atopy history		
Present	12	41.4
Absent	17	58.6
Maternal prophylactic antibiotic use		
Yes	29	100.0
No	0	0.0
Type of antibiotic		
Ampicillin	10	34.5
Ceftriaxone	1	3.5
Metronidazole	3	10.3
Cefazoline	15	51.7

Table 2 | **Skin bacteria distribution between preterm and term neonates.**

Bacterium	Total		Gestational age			
			Preterm		Term	
	n	%	*n*	%	*n*	%
Staphylococcus haemolyticus	8	27.6	4	26.7	4	28.6
Staphylococcus aureus	2	6.9	2	13.3	0	0.0
Staphylococcus epidermidis	2	6.9	2	13.3	0	0.0
Staphylococcus hominis	9	31.0	8	53.3	1	7.1
Staphylococcus equorum	1	3.4	0	0.0	1	7.1
Staphylococcus cohnii	3	10.3	2	13.3	1	7.1
Staphylococcus lentus	2	6.9	2	13.3	0	0.0
Staphylococcus warneri	1	3.4	1	6.7	0	0.0
Staphylococcus sciuri	1	3.4	0	0.0	1	7.1

Discussion

This study found that term newborns exhibited predominantly acidic skin pH, whereas preterm infants tended to have alkaline skin pH. Of the 16 newborns, 12 presented with alkaline skin pH, and only three of the 13 term newborns showed similar findings. These findings support our initial hypothesis and are consistent with previous work by Marissen et al., who reported that preterm newborns generally exhibit alkaline skin (5). Several structural differences between preterm and term skin may explain these findings. Term newborns typically have a total skin thickness of approximately 1.2 mm, compared to 0.9 mm in preterm infants. The epidermis in term newborns averages 40 to 50 µm in thickness with a stratum corneum of 9 to 10 µm, whereas in preterm newborns the epidermis is thinner, about 20 to 25 µm, and the stratum corneum is only 4 to 5 µm thick (19). Because the stratum corneum plays a key role in skin acidification through NHE-1 activity, a thinner barrier likely reduces H^+ concentration and results in a more alkaline skin environment (8).

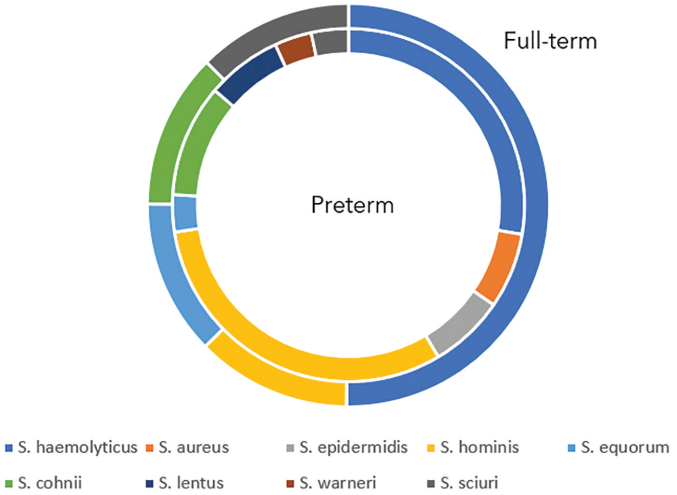

Figure 1 | Distribution of bacterial species in preterm and full-term newborns.

Table 3 | Skin pH and *Staphylococcus* spp. colonization between preterm and term newborns.

Parameters	Preterm (*n* = 16)		Term (*n* = 13)	
	n	%	*n*	%
pH				
Acid	4	28.6	10	71.4
Alkaline	12	80.0	3	20.0
Staphylococcus spp. swab				
Positive	13	61.9	8	38.1
Negative	3	37.5	5	62.5

Table 4 | *Staphylococcus* spp. colonization count comparison by skin pH.

pH	Positive swab	Mean	*SD*	Median	(Min–max)
Alkaline	14	82.14	106.99	27.50	3.00–300.00
Acid	7	6.29	10.19	2.00	1.00–29.00

SD = standard deviation.

Another possible mechanism involves natural moisturizing factor (NMF), which is reduced in preterm infants due to increased transepidermal water loss (TEWL). Mathanda et al. reported significantly higher TEWL in preterm newborns compared to term infants (20). Visscher et al. further showed that filaggrin levels, the precursor of NMF, are lower in preterm newborns (21). NMF provides acidic components such as urocanic acid, pyrrolidone carboxylic acid, lactic acid, and free amino acids, all of which contribute to the acid mantle (22). Reduced NMF in preterm newborns disrupts acidification, thereby contributing to elevated skin pH. Maintaining an acidic stratum corneum is essential for barrier integrity and antimicrobial defense. Multiple mechanisms, including NHE-1 antiport activity, phospholipase A2–mediated release of free fatty acids, and histidase-mediated conversion of histidine to urocanic acid, work synergistically to maintain this acid balance (23). Alkaline pH interferes with these pathways, impairs lipid-processing enzymes such as β-glucocerebrosidase and acid sphingomyelinase, and ultimately reduces ceramide levels (11). Lower ceramide impairs the physical barrier and increases susceptibility to microbial invasion (24).

In our cohort, the mean colony-forming unit (CFU) count was substantially higher in newborns with alkaline skin compared to those with acidic skin. Alkaline conditions may promote colonization by enhancing serine protease activity, such as kallikrein 5 and 7, which disrupt desmoglein-1 and compromise stratum corneum cohesion (10). In parallel, alkaline pH reduces antimicrobial peptide activity, including dermcidin, further facilitating pathogen growth (25).

Conclusions

In conclusion, skin pH and *Staphylococcus* spp. colonization differed between preterm and term newborns. This study has several limitations. First, it was conducted at a single center, which may limit the generalizability of the findings; future studies should therefore include multicenter settings. Second, no longitudinal follow-up was performed to assess the potential development of dermatological conditions later in infancy. Future research should incorporate follow-up at 2, 6, and 24 months of age to evaluate possible long-term skin alterations. Third, this study included

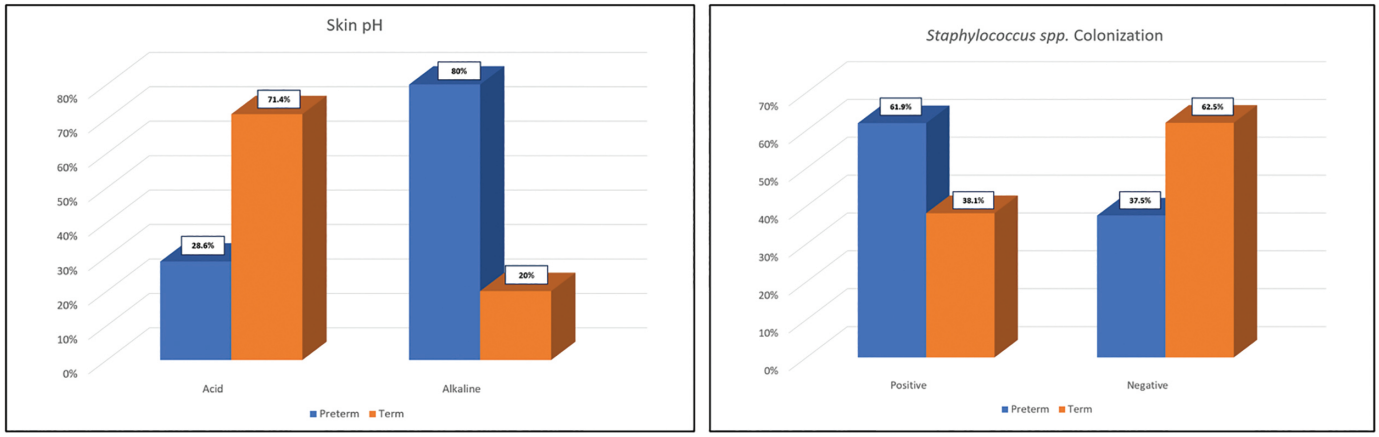

Figure 2 | Skin pH and *Staphylococcus* spp. colonization comparison.

only newborns delivered by cesarean section. Further studies should compare skin pH and *Staphylococcus* spp. colonization between infants born via cesarean and vaginal delivery.

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