Received: 30 April 2020 | Revised: 9 September 2020 | Accepted: 11 September 2020

DOI: 10.1111/exd.14198

REGULAR ARTICLE

Experimental Dermatology WILEY

Characterization of the microbiome in the infant diapered area: Insights from healthy and damaged skin

Amber Teufel | Brian Howard | Ping Hu | Andrew N. Carr

The Procter and Gamble Company, Cincinnati, OH, USA

Correspondence
Amber Teufel, The Procter and Gamble Company, Cincinnati, OH, USA.
Email: teufel.ag@pg.com

Funding information
This study was fully funded by Procter & Gamble.

Abstract

It has been recognized for nearly a century that human beings are inhabited by a remarkably dense and diverse microbial ecosystem, yet we are only just beginning to understand and appreciate the many roles that these microbes play in human health and development. Establishment of the microbiome begins at birth, but many previous studies on infant skin health have focused on Candida species. Little is known on the full microbial composition across different areas and even less is known on how these communities change during disease/inflammatory states. In this clinical study, infants were recruited during periods of diaper dermatitis (DD) and health to characterize the skin microbiome in these two states. Substantial shifts in the skin microbiome were observed across four sites in the diapered area (genitals, intertriginous, buttocks and perianal), as well as during periods of DD. As DD scores increased, there was a shift in relative abundance that demonstrated higher community percentages of faecal coliforms, such as *Enterococcus*, and lower percentages of *Staphylococcus* strains. In high-rash samples, the predominant *Staphylococcus* species is *S aureus*, potentially implicating *S aureus* as a DD aetiological agent. This study provides new information related to the microbiome on infant skin in the diapered area and provides insights into the role of the microbiome in the development of DD.

KEYWORDS
Candida, diaper rash, infant, microbiota, Staphylococcus

1 | INTRODUCTION

The skin microbiome is a complex ecosystem that consists of numerous different communities all working together to protect against infection. The initial colonization of an infant's skin is critical in cutaneous homeostasis and contributes significantly to a strong skin immune system.[1] It is only after birth that environmental microbiota begins to colonize the stratum corneum transforming it into a complex community that must change with the environment. The skin is in a constant state of change as it adapts to changes from a wet (in utero) to dry environment, skin hydration, lowering of skin pH, sebaceous activity and temperature, all of which contribute to rapid epidermal cell proliferation. The changing and evolving skin of infants also indicates a rapidly changing and evolving infant microbiome that may play an important role in the development of skin-related disorders in an infant's life.

All authors are full-time employees at Procter & Gamble.

Preliminary data from this study were presented in part at the 13th World Congress of Pediatric Dermatology Conference, 6-9 July 2017: Chicago, IL USA.

The human microbiome is an evolving area of research, and its impact on health and wellness has only recently been appreciated. Despite the advances over several decades, our understanding of the complex interplay between the host and microbiome and how it might impact disease onset or resolution is not yet well understood. It is well known that the microbiome can vary dramatically based on anatomical location, in part driven by the specific microenvironment.[2-5] In the developing infant, the diapered area represents a unique environment as it is covered most of the day, in contact with a diapering product, and accompanied by cleaning and/or bathing procedures.[6] The extreme conditions within the diaper and the unique microclimate (eg temperature and relative humidity) would indicate that the microbiota within the diaper area is likely significantly altered compared to other parts of the body, and any alteration in the microbiome could impact skin properties (eg, skin pH) and the development of DD.

Diaper dermatitis, also known as "diaper rash" or "nappy rash," is an acute, inflammatory dermatitis within the diapered area subsequent to a breach of the stratum corneum and is characterized by the presence of erythema, oedema, papules and/or pustules. DD is one of the most frequent skin ailments in infants and is a source of pain and discomfort.[7] Rates of DD in the literature are highly variable (16%-65%), which may reflect difficulty in assessing or reporting the condition as most cases are treated by the caregiver.[8-11] As such, DD visibly resolves within a few days, but can be prolonged if associated with infection. The causes of DD are multifactorial and include overhydration of the skin, friction and exposure to irritants in the urine and faeces, including pH-sensitive enzymes that can degrade the skin over time.[12-16] Eventually, almost every baby will have an episode of DD, especially when the baby begins eating solid foods, thus changing the acidity of the bowel movement.

Dermatitis can come in many forms, but when microbial in origin, the most commonly associated organisms are *Candida* or *Staphylococcus*. Yeast vs bacterial infections often present with different symptoms and in different locations as Candida infections often present with erythematous and scaly plaques involving the folds, with satellite papules or pustules while perianal streptococcal dermatitis demonstrates persistent localized erythema with itching after defecation.[17] These infections are thought to result after damage to the skin has occurred, although this is difficult to determine in each case.

A recently published study[18] found differences in the incidence and severity of diaper rash across four anatomical sites (perianal, intertriginous (leg folds), genitals, buttocks) with the lowest DD associated with the lowest skin pH and relative humidity in the diaper. This is not too surprising given the genital area is exposed to the highest levels of urine/wetness which can cause large changes to the pH and moisture levels in this area selecting for acidophiles. The perianal space is a site of skin-to-skin contact and is most commonly exposed to faeces and thus a site where faecal coliform bacteria and intestinal yeast and moulds tend to be most prevalent. The intertriginous regions (leg folds) are characterized by skin-to-skin contact that results in higher moisture, lower oxygen availability and increased friction. Intertrigo is a type of inflammatory rash that occurs within the body folds, which are more susceptible to exposure of bodily secretions like sweat, urine or faeces,[19] and is often infected with bacteria such as *Corynebacterium* and *Staphylococcus* and fungi such as *Candida*.[20] Finally, the buttock region is the largest area represented and is the most exposed to air and tends to be drier than the other regions within the diaper. The increase in oxygen availability often selects for aerobic microorganisms that can withstand the changing temperatures and tend to be part of the normal skin microbiota. This concept is supported by previous data showing the presence of *Candida* yeasts in some, but not all instances of DD. The full microbial community has yet to be characterized, both in terms of anatomical site and/or during episodes of DD.[21] In any case, the differences in the DD prevalence and severity within the diapered region require additional studies to understand possible contributors, including the role of the microbiome.

The role of microorganisms in DD is often only considered in the cases of stratum corneum damage wherein organisms can penetrate to deeper layers and cause clinical infection. As microbial identification tools such as sequencing and qPCR have advanced, the role these microbes play in dermatitis is becoming more well established and the data herein provide additional learning on the associations and provide new hypotheses on the possible aetiology of DD. However, the infant microbiome is constantly changing as the baby is exposed to new foods, new environments and new antibiotics. With each introduction, the microbiome changes, creating difficulties in the study of their role in disease state and complicating efforts to understand establishment of an infant's typical microbiome.

The objective of this study was to characterize bacterial and fungal communities within the four main areas of the diaper region (genital, intertriginous, perianal and buttocks) during various stages of DD.

2 | METHODS

2.1 | Clinical design

The study was a 4-week longitudinal surveillance study and included 18 babies, ages 5-15 months, with at least one moderate rash event in the past 2 months prior to study start and at least 1 episode of rash during the 4-week study period. Babies were predominantly non-Hispanic (89%), White/Causasian (83%), equally split by sex (50%) with a mean age of 10.6 months (Table 1). Three to 7 days before enrolment, babies were assigned to a standard diaper and wipe product to be used according to their existing habits. Parents were asked to avoid use of any topical skin product during the study period. Other inclusion criteria: full-time use of disposable diapers, child not to be bathed the morning of evaluation, child regularly has at least 2 bowel movements/day. Exclusion criteria: Chronic medical condition requiring ongoing medication, chronic dermatological condition, have taken any systemic or topically applied antifungal, antibacterial, steroid or antihistamine medication, has ever exhibited hypersensitivity to topically applied skincare products.

Microbial swabs were taken at the genitals, intertriginous (leg folds), perianal region and buttocks after the regions were graded for rash by a trained grader[18] Rash grading was completed using a validated grader scoring tool. The tool categorizes DD on a 7-point scale (0-3, with 0.5 increments), assessing skin redness, degree of skin coverage and presence of papules and/or pustules.[22] For the purpose of this study analysis, rash was divided into no rash (grade 0), low rash (0.5-1.0) and high rash (1.5+). Swabs were collected by trained clinical technicians with appropriate personal protective equipment as per the clinical protocol and stored at −80°C until processing. Samples were then processed at a contract facility (Research and Testing Laboratories). It is important to note that samples were collected from multiple time points from a single subject in rash and no-rash conditions (Table S1).

2.2 | DNA isolation and sequence analysis

The sequence dataset has been deposited on the NCBI SRA Database with Bioproject ID: PRJNA656308. Bacterial DNA was isolated from each swab using commercial kits available at the time (MoBio Powersoil DNA kit). 16S/ITS 454 Pyrosequencing was used to sequence the V1-V3 region of bacterial 16S gene and the ITS region of fungal rRNA gene. Sequence reads and complete datasets can be found in Tables S2 and S3 (bacterial and fungal, respectively). Several controls were included in the analyses and the negative controls did not yield enough reads for analysis in this dataset. Standard data analysis tools for denoising, chimera and quality checks and QIIME1.9 were used to obtain taxonomic identification for microbiome profiling using GreenGene version gg_13_8 database and UNITE ITS version 8.0 Database. Many samples sent for ITS sequencing did not yield enough reads and this was more common in exposed areas like the buttocks. It is likely that exposed skin such as the buttocks provided a more hostile environment to fungi and thus less fungi were recovered as opposed to areas such as the intertriginous zones which retain high humidity and debris due to the skin-to-skin contact and had higher relative abundance overall. To ensure quality, ITS sample with <525 high-quality reads (threshold selected based on negative control reads count) was removed from analysis. QIIME1.9 UCLUST method was used to establish clusters and taxonomic identification. All sequences from all samples were clustered into operational taxonomic units (OTUs) based on their sequence similarity. Sequences were clustered at 97% with UCLUST, with each resulting cluster represented at a species level (QIIME). Thus, species classification reported is an indication of possible species detection based on the uclust model and current techniques for picking OTUs.

TABLE 1 Demographics

Study Demographics N = 18	
Measures	**Result**
Ethnicity	
Hispanic/Latino	2 (11.1%)
Not Hispanic or Latino	16 (88.9%)
Race	
White/Caucasian	15 (83.3%)
Multiracial	3 (16.7%)
Gender	
Female	9 (50.0%)
Male	9 (50.0%)
Age (months)	
Mean	10.6
Median	12.0
Min-max	5.0-15.0
Fitzpatrick score	
I	1 (5.6%)
II	10 (55.6%)
III	4 (22.2%)
IV	2 (11.1%)
V	1 (5.6%)
Weight (kg)	
Mean	9.3
Median	10.0
Min-max	6.8-10.9

2.3 | Statistics

Unpaired two-sample Wilcoxon test was performed to compare high-rash and low-rash differences. Both Pearson and Spearman correlation analysis was used to identify association of microbial genre association with DD. Kruskal-Wallis test was used to identify site-or rash-associated microbial changes. Bar plots were generated using OTU relative abundances with R statistical software. Shannon, Simpson, Chao index and Bray–Curtis distance were also calculated and demonstrated significant bacterial differences among sites ($P = 3.16e\text{-}21$) and significant fungal differences in rash vs non-rash sites ($P = .03$). Unweighted Unifrac analysis for the bacterial population was also calculated by site and rash severity (Figure S1).

3 | RESULTS

3.1 | Bacterial composition by anatomical region

Microbial analyses of swabs taken from four anatomical sites within the diapered area (genitals, intertriginous, buttocks and perianal) revealed distinct microbial communities with relative abundance of *Staphylococcus* strains being the highest in the intertriginous and genital regions and faecal bacteria, such as *Bacteroides* and *Faecalibacterium*, being higher in the perianal and buttock regions (Figure 1). In the intertriginous region, *Staphylococcus* accounted for 54.08% of the community diversity with other contributors

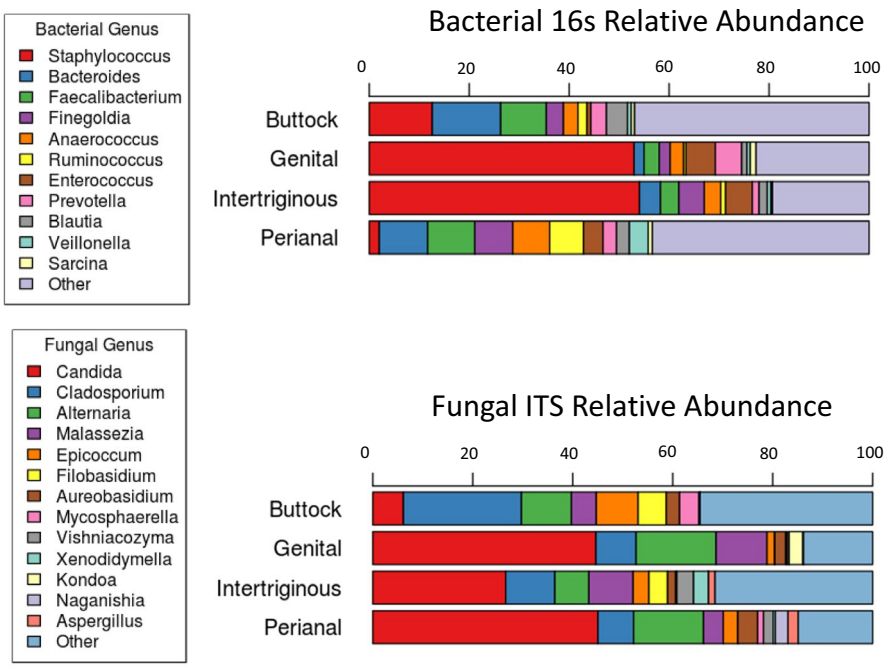

FIGURE 1 Baby diapered area microbiome profile—genus level. Fungal and bacterial relative abundance is demonstrated by region within the diapered area from which the sample was taken' perianal, buttock, genital and intertriginous zones

including *Enterococcus* (5.34%), *Finegoldia* (5.11%) and *Bacteroides* (4.20%). Similarly, *Staphylococcus* also accounted for the majority of the community in the genital region (53.00%), followed by *Enterococcus* (5.88%) and *Prevotella* (5.27%). The buttock and perianal regions are comprised of many lower-abundance organisms with no one genus accounting for more than 14% of the community. Buttock and perianal regions had significantly higher diversity than genital and intertriginous sites (Shannon diversity[23]: buttock 5.59, perianal 5.31, intertriginous 3.88, genital 3.77, p value of Kruskal-Wallis[24] test $3.16E^{-21}$). In the buttock region, the highest relative abundances were *Bacteroides* (13.70%), *Staphylococcus* (12.61%) and *Faecalibacterium* (9.13%). *Bacteroides* was also the most abundant genus in the perianal region (9.68%), followed by *Faecalibacterium* (9.45%), *Finegoldia* (7.60%) and *Anaerococcus* (7.37%).

We also sought to characterize the microbiome at the species level (Figure 2 [see Methods section and study limitations]). The *Staphylococcus* genus previously identified was subdivided into three species: *S haemolyticus*, *S aureus and S epidermidis*. The predominant *Staphylococcus* species varied by location with *S aureus* predominant in the buttock region (4.32% of all recovered species, second to *Faecalibacterium prausnitzii* at 9.13%), *S haemolyticus* in the genital region (16.52% overall) and *S epidermidis* in the intertriginous region (14.92% overall). The perianal region had low *Staphylococcus* abundance across all species (below the top 10 most highly abundant species) instead being dominated by *Faecalibacterium prausnitzii* (9.45%) and *Ruminococcus gnavus* (5.81%).

3.2 | Fungal composition by anatomical region

Fungal communities also demonstrated distinct differences in community composition within each of the four diapered regions examined (Figure 1). We did not observe significant fungal diversity differences among different regions, although the buttock region had the highest diversity (Shannon Diversity 1.98) and the perianal region had the lowest (Shannon Diversity 1.41). *Candida* was more abundant in occluded areas (skin-to-skin contact) such as the perianal (45.03%) and intertriginous regions (26.59%), but also at the genitals (44.62%), with much lower abundance in the buttock region (6.13%). In the less occluded area, the buttocks, the most abundant genus was *Cladosporium* (23.61%). Some genera were found in all areas such as *Alternaria* which comprised 6%-15% and *Malassezia* which was 3%-10% of each population.

Fungal abundance was also assessed by species-level determinations (Figure 2) and the predominant species identified in the genital, intertriginous and perianal regions was *Candida albicans* (43.27%, 23.64%, 29.17%, respectively). The buttock region samples identified three more prevalent species: *Cladosporium delicatulum* (23.06%), *Alternaria betae-kenyensis* (10.00%) and *Epicoccum nigrum* (8.32%) highlighting the buttock region as a site with low-*Candida* biome.

3.3 | Bacterial composition by DD severity

As DD scores increased, the overall bacterial diversity remained unchanged while there was a downward shift in the relative abundance of *Staphylococcus* strains (34.05% no rash, 22.75% high rash) and an upward shift in faecal-associated bacteria such as *Enterococcus* (2.59% low rash, 11.11% high rash) (Figure 3). Interestingly, community composition at low- and high-rash scores differed among the sites but followed the same principles of high-rash communities having more *Enterococcus* and lower *Staphylococcus* compared to no-rash communities. The perianal region community was more evenly dispersed compared to the other regions and demonstrated

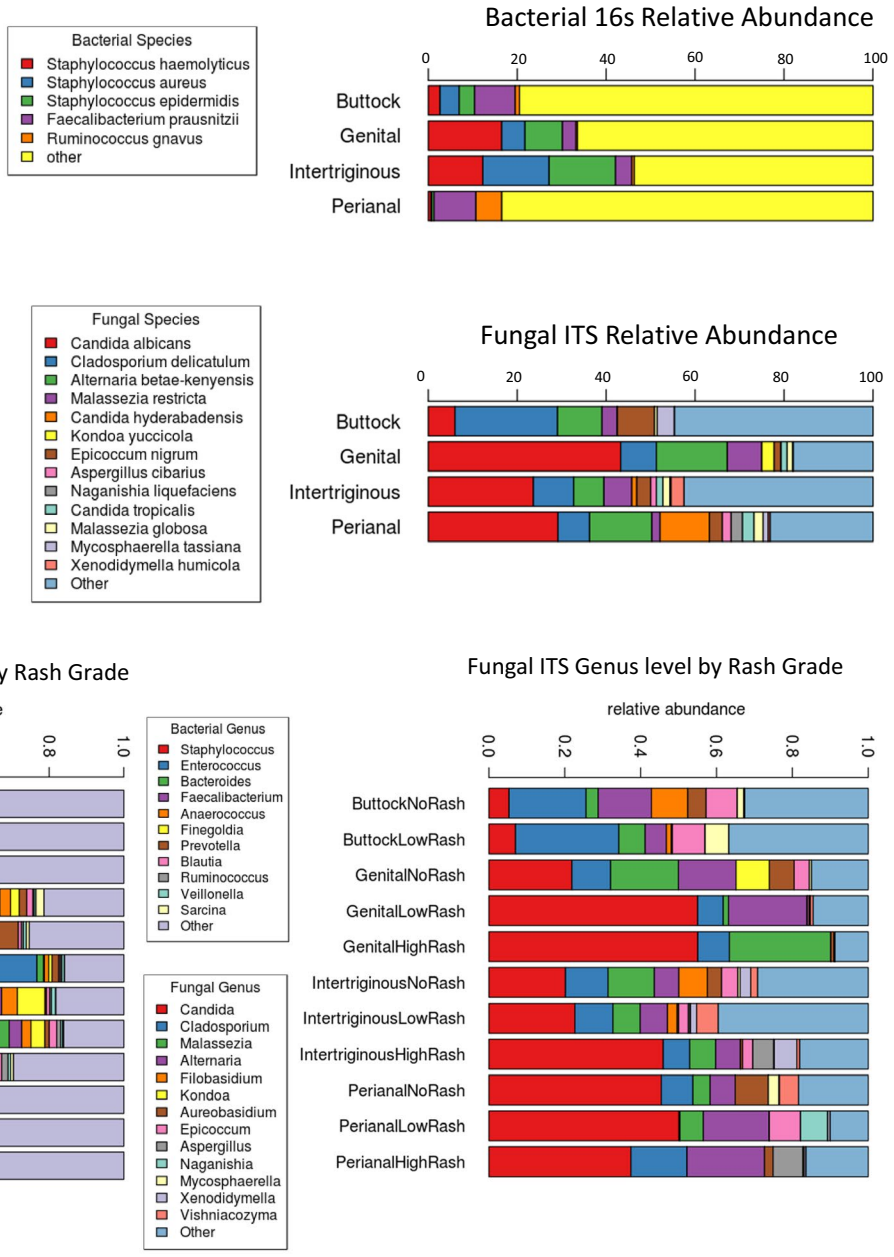

FIGURE 2 Baby diapered area microbiome profile—species level. Fungal and bacterial relative abundance is demonstrated by region within the diapered area from which the sample was taken' perianal, buttock, genital and intertriginous zones

FIGURE 3 Baby diaper rash microbiome profile—genus level. Fungal and bacterial relative abundance is demonstrated by the level of rash each infant experienced: no rash, low rash or high rash

low-rash communities as having more *Anaerococcus* and *Finegoldia* compared to high-rash communities.

The overall abundance of each bacterial species community member was also analysed in no-rash and high-rash samples (Figure 4). The current data analysis suggests the major *Staphylococcus* species for diapered areas includes *S epidermis*, *S aureus and S haemolyticus*. The abundance of *S aureus* was larger during high-rash state (9.52%) than low-rash state (5.02%), while the abundance of *S haemolyticus* was lower during episodes of high rash (4.63%) vs low rash (9.71%). During high-rash episodes, *Enterococcus*, known to be a faecal coliform, was the second most abundant organism and was found at higher abundance compared to low-rash states, regardless of anatomical site.

3.4 | Fungal relative abundance by DD severity

In the absence of rash, the steady-state communities were more evenly distributed between *Candida* (19.91%), *Cladosporium* (13.46%) *Alternaria* (10.19%) and all other genera. Multiple genera were either identified only in the no-rash samples or in low abundance otherwise, such as *Kondoa*, *Filobasidium*, *Vishniacozyma* and *Mycosphaerella*. *Candida* increases correlated with increased rash and other organisms such as *Malassezia* and *Cladosporium* remained relatively constant regardless of score (*Malassezia* 5.71%-8.96% and *Cladosporium* 9.37%-13.46%). As DD scores increased, the fungal community shifted as *Candida* abundance increased from 19.91% of the no-rash communities to 45.18% of the high-rash sites (Figure 3, Figure 4).

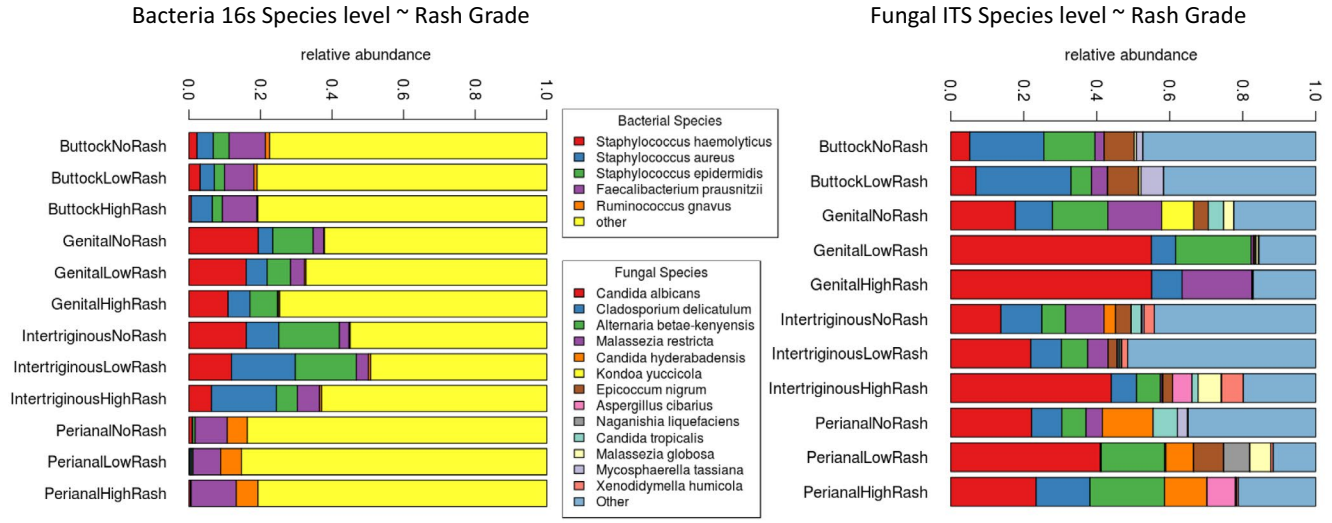

FIGURE 4 Baby diaper rash microbiome profile—species level. Fungal and bacterial relative abundance is demonstrated by the level of rash each infant experienced: no rash, low rash or high rash

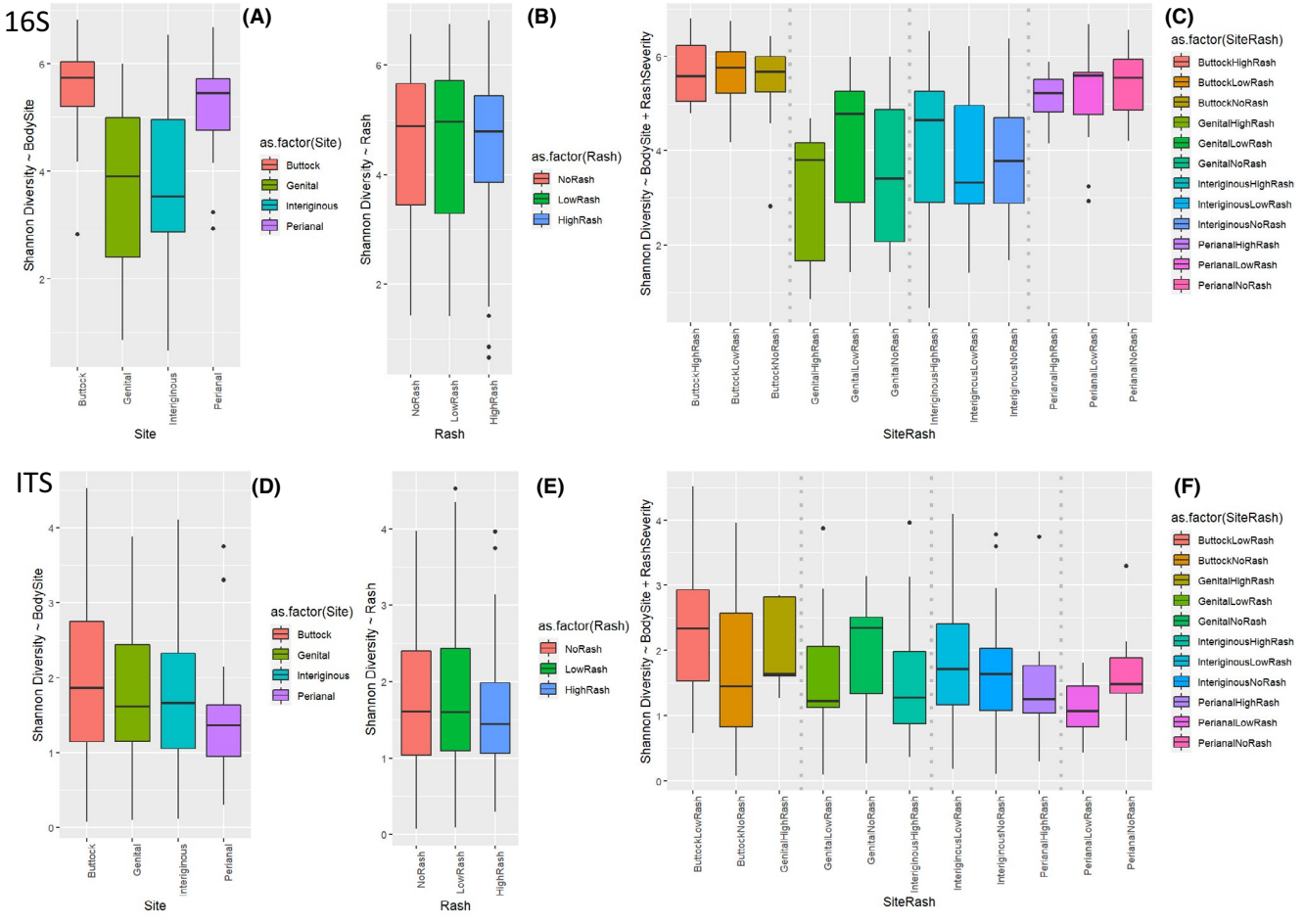

FIGURE 5 Shannon diversity scores for 16s was classified by site (Panel A: Kruskal Wallis chi-squared = 98.67, *P*-value <2.2e-16), rash severity (Panel B: Kruskal Wallis chi-squared = 0.583, *P*-value =0.747), and both site and rash (Panel C: Kruskal Wallis chi-squared = 104.5, *P*-value <2.2e-16). Shannon diversity scores for ITS was classified by site (Panel D: Kruskal Wallis chi-squared = 6.258, *P*-value =0.099), rash severity (Panel E: Kruskal Wallis chi-squared = 0.984, *P*-value =0.611), and both site and rash (Panel F: Kruskal Wallis chi-squared = 19.39, *P*-value =0.036).

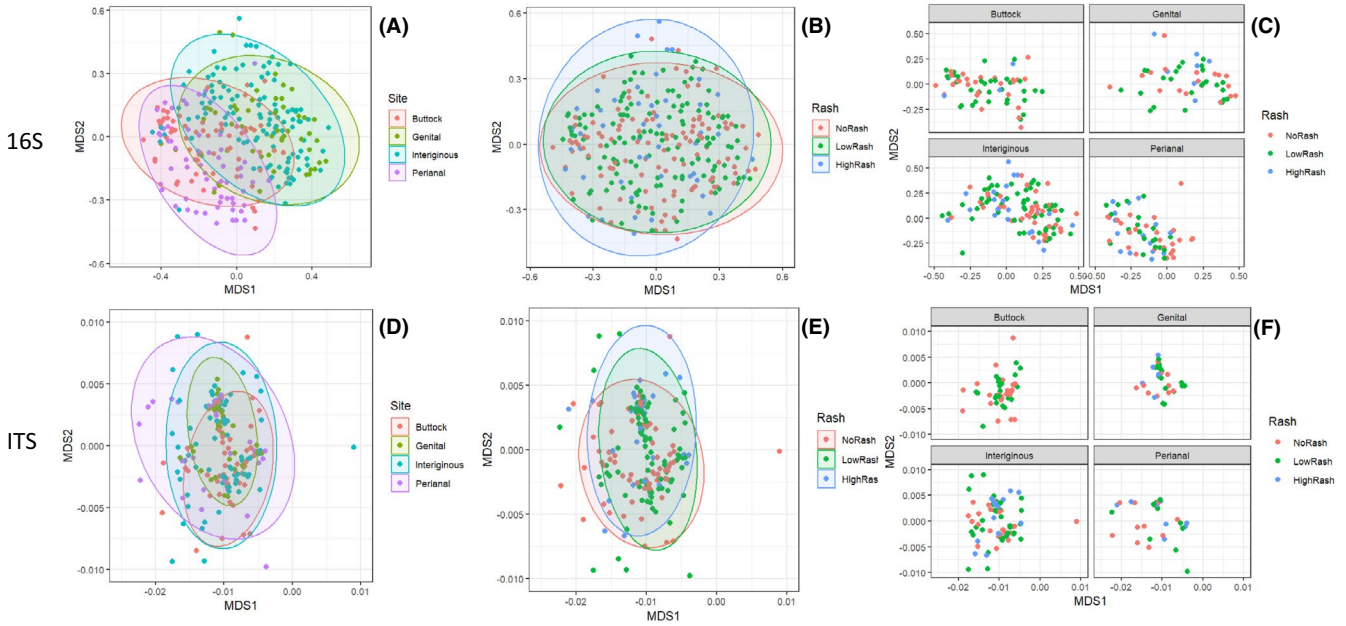

FIGURE 6 Bray-curtis distance by subject for 16s and ITS among site, rash severity and site/rash. 16s Beta diversity was measured using Bray-Curtis distance by site (Panel A: *P*-value =.001), rash severity (Panel B: *P*-value =.006) and by site/rash (Panel C: *P*-value =.001). ITS Beta diversity was measured using Bray-Curtis distance by site (Panel D: *P*-value =.001), rash severity (Panel E: *P*-value =.005) and by site/rash (Panel F: *P*-value =.001)

Additionally, organisms such as *Aspergillus* and *Lasiosphaeriaceae* were only identified in reportable numbers in the high-rash samples. *Candida* showed significant positive correlation with diaper rash severity ($P = .01$). *Candida albicans* was identified as the predominant species and significantly correlated with diaper rash severity ($P = .01$). The association of high *C albicans* relative abundance with high-rash grades was most prevalent in the intertriginous region.

3.5 | Alpha and beta diversity

Additionally, alpha and beta diversity was determined using Shannon (Figure 5) and Bray-Curtis (Figure 6) analysis for both the 16s and ITS populations. Overall, lower 16s alpha diversity was observed on genital (Shannon 3.77) and intertriginous (Shannon 3.88) regions, likely due to the higher moisture content in this area. Conversely, the buttock (Shannon 5.59) and perianal (Shannon 5.31) regions had the highest 16s alpha diversity, presumably due to the buttock skin being exposed to lower humid, higher oxygen conditions (Kruskal-Wallis chi-squared =98.567, $df = 3$, *P*-value < 2.2e-16). Rash severity did not result in statistically significant changes in 16s alpha diversity (Kruskal-Wallis chi-squared =0.583, $df = 2$, *P*-value =0.747). Fewer differences were observed in alpha diversity of the ITS population with all four regions presenting with similar Shannon scores (buttock 1.98, genital 1.76, intertriginous 1.75 and perinanal 1.41 (Kruskal-Wallis chi-squared =6.258, $df = 3$, *P*-value =.099)). Rash severity did not result in statistically significant changes in ITS alpha diversity (Kruskal-Wallis chi-squared =0.984, $df = 2$, *P*-value =.6114). Beta diversity scores were calculated using Bray-Curtis distance and demonstrated statistically significant changes in both the 16s and ITS populations by both site and rash severity (Adonis test *P*-value by site =.001 for both 16s and ITS: Adonis test *P*-value by rash severity 0.006 for 16s and 0.005 for ITS).

4 | DISCUSSION

Diaper dermatitis is an extremely common rash that appears on the skin under the diapered area, and it is unknown whether the microbial balance within the diapered region can contribute to DD. This study found that the infant skin microbiome varies by anatomical site within the diapered area and with changes in skin health. In terms of anatomical regions, the genital and intertriginous regions maintain similar bacterial communities (dominated by *Staphylococcus*), while the buttock and perianal regions are dominated by *Bacteroides*, *Faecalibacterium* and numerous other low, but equally abundant community members. *Staphylococcus* is a facultative anaerobe that grows by aerobic respiration or by fermentation that yields lactic acid[25] and can grow and replicate in any human tissue including the organs like the skin. These growth features likely contribute to their presence in the genital and intertriginous regions that may have lower oxygen availability or episodic exposure to urine and its components. The *Staphylococcus* species also varied by location as the opportunistic organism, *S aureus*, was prevalent in the buttock region, likely due to exposure of faeces and oxygen availability, while normal skin microbiota species like *S epidermidis* and *S haemolyticus* were in higher abundance the genital and intertriginous regions which have lower, less frequent exposures to faecal material.

Interestingly, no *Staphylococcus* species was highly abundant in the perianal region where we might expect to see *S aureus*, but instead was dominated only by faecal coliforms such as *Faecalibacterium*. *Bacteroides* and *Faecalibacterium* are both members of the indigenous intestinal microbiota in humans where they contribute to normal intestinal development, physiology and function. They often survive outside the intestinal tract due to their extended aerotolerance, simple nutritional requirements and ability to utilize diverse substrates.[26] Their higher abundance in the buttock and perianal region can be expected due to the higher levels of faeces present in these two areas compared to the rest of the diapered region. Their presence also suggests they were retained in areas of high faecal and high diaper rash during normal hygienic practices (eg bathing, cleaning of a bowel movement).

A recent study in China investigated the microbiota of infant skin both on healthy (31 babies) and those during an episode of DD (54 babies).[27] The microbiota of healthy skin was dominated by *Staphylococcus*, *Anaerococcus*, *Finegoldia*, *Corynebacterium*, *Streptococcus* and *Clostridium*. Similar to our results, the highest average abundance belonged to *S epidermidis* and *S haemolyticus*. However, in the current study, four regions were examined across the diapered area as opposed to a single area (ie, buttocks) in the previous investigation. Our results demonstrated the highest relative abundance of *S haemolyticus* (followed by *S epidermidis*) only in the genital and intertriginous zones and *Faecalibacterium prasnitzii* dominated in the buttock and perianal regions.

As previous research would suggest, *Candida* was the predominant organism isolated across diapered region as *Candida* and *Malassezia* are well-known commensal organisms on the skin.[28] More specifically, *C albicans* was the most predominant species in the genital, intertriginous and perianal regions likely due to their occluded and moist environments. *Candida* is an opportunistic pathogenic yeast that is a common member of the natural gut microbiome and can survive well outside of the human body and is often considered commensal. Due to its ability to respond to environmental conditions, it has three morphological forms that change based on temperature, pH and nutrient availability and make the organism especially hard to remove from the skin. Warmth and moisture that can occur within the genital, intertriginous and perianal regions facilitate infiltration of the stratum corneum by *Candida* species[29] and the higher heat, humidity and CO_2 levels then increase skin's susceptibility to infection with dermatophyte microconidia or *Candida* spores.[30] This is consistent with previous work demonstrating a reduction in the incidence of Candida infections in the diapered area by the use of more "breathable" diapers that are designed to improve air exchange and reduce humidity within the diaper.[21]

In addition to the regional microbiome differences, there were also shifts in each of these communities based on the level of DD present. As DD scores increased, there was a shift in relative abundance that demonstrated higher community percentages of faecal coliforms, such as *Enterococcus*, and lower percentages of *Staphylococcus* strains. In high-rash samples, the predominant *Staphylococcus* species is *S aureus*, potentially implicating *S aureus* as a DD aetiological agent. Staphylococcus has been implicated previously in severe DD where the bacteria produce a toxin (staphylococcal exfoliative toxin A) that reddens and damages the skin and can cause low-grade fevers.[31] In low-rash communities, the dominant species, *S epidermidis* which was mentioned previously, is part of the normal skin microbiota. The most prevalent fungal member of the community in high-rash samples was *Candida* which corresponds to previous observations.[31,32] Overall, fewer fungal sequences were recovered from baby skin, with the least sequences observed from healthy skin. Fungal cells are known to be harder to break than bacterial cells using the methods demonstrated here, and thus, DNA extraction could lead to more bacterial DNA being observed than fungal DNA. In terms of locations, it is possible that the low humidity on the buttock region led to lower fungal abundance compared to that of the intertriginous or genital regions where folded skin allows for moisture and debris retention that may be favourable for fungal growth.

The study by Zheng et al[27] examined DD samples and found higher intragroup similarities in community composition in the healthy samples compared to the DD samples. Similar to our results, the DD samples were prevalent in the *Staphylococcus* genus (predominantly *S haemolyticus* and *S epidermidis*) followed by *Pseudomonas* and *Ruminococcus* (*R gnavus*). In our study, it is interesting to note that there were only nine species that differed between the healthy and DD groups implying that the natural microbiota is important in the regulation of disease state. The pathophysiology of diaper rash is not clearly defined but likely results from a combination of factors that includes wetness, friction, urine and faeces, and the presence of microorganisms. It is unclear whether the relative abundance of microorganisms on the skin plays a direct role in causing DD or perhaps these abiotic factors create a hostile environment that increases the susceptibility of the skin to damage and secondary infection and subsequent inflammation, characteristic of DD. One such mechanism could include alterations in the acidic nature of the skin surface which is essential for maintaining normal microbiota and provides innate antimicrobial protection against the invasion of pathogenic yeasts and bacteria. A consequence of this could be alterations in the skin pH which could impact the activity of various pH-sensitive enzymes in faeces or outer layers of the skin, increasing the likelihood of skin damage.[33]

This study shows that the diapered environment is unique and that "microenvironments" exist within the diaper locations in both healthy and DD skin. This could be the result of episodic increases in moisture and pH during urination events. Increases in skin wetness can be utilized by fungi, like *Candida* while the mixing of higher pH urine with faecal material can activate pH-dependent protease, lipase and/or urease activity[16] and subsequent inflammation/infection which underlie diaper dermatitis. In this study, *Candida* appears more abundantly in the moist, occluded areas of the skin (intertriginous leg folds, genitals and perianal regions) and less so in the relatively drier buttock region. Further, the intertriginous folds showed the highest rate of increase in *Candida* associated with moderate-to-severe DD. Previous studies demonstrated that the number of *Candida* yeasts in the diapered area without dermatitis is low and is isolated in less than

4% of cases, whereas 70%-92% of children with DD have measurable *Candida*.(34) Overall, the most statistically significant organisms related to diaper rash were *C albicans* and *S aureus*, supporting the concept that the microbiota play an important role in DD disease state.

This study provides new foundational information characterizing the microbiome in the diapered area under healthy and DD states. These data are also important to better understand the impact of episodic exposes to urine and faeces in the context of caregiver behaviours on microbiome relative abundance. The infant skin microbiome varies by anatomical site within the diapered area as well as with changes in skin health (DD) and implies potential biological triggers for DD and potential opportunities for prevention and treatment. Additional studies are needed to further delineate the role of the variety of factors that could impact skin health in the diapered area.

It is important to acknowledge limitations in the current study. The sample size in the present investigation does not allow a robust analysis of the skin microbiome with regard to diet, which could be a contributing factor to the difference between subjects. Further, while we report data at the species level, the techniques employed herein are not definitive in making such characterization and the findings should be confirmed in a future investigation, using more robust techniques (eg, metagenomics).

CONFLICTS OF INTEREST

The authors are full-time employees at Procter & Gamble. This study was funded by Procter & Gamble.

AUTHOR CONTRIBUTIONS

BH helped designed the research study and performed the research; AC, PH and AT analysed the data; and AT, BH, PH and AC wrote the manuscript.

DATA AVAILABILITY STATEMENT

The sequence dataset has been deposited on the NCBI SRA Database with Bioproject ID: PRJNA656308.

ORCID

Amber Teufel https://orcid.org/0000-0001-6752-8684
Andrew N. Carr https://orcid.org/0000-0003-1676-8033

REFERENCES

[1] A. M. Schneider, A. M. Nelson, *Ped Dermatol* **2019**, *36*, 815.
[2] K. A. Capone, S. E. Dowd, G. N. Stamatas, J. Nikolovski, *J Investig Dermatol* **2011**, *131*, 2026.
[3] J. Segre, H. Kong, O. Tirosh, A. Byrd, *FASEB J* **2019**, *33*;93.
[4] C. M. Timm, K. Loomis, W. Stone, T. Mehoke, B. Brensinger, M. Pellicore, P. P. Staniczenko, C. Charles, S. Nayak, D. K. Karig, *Microbiome* **2020**, *8*, 1.
[5] P. A. Dimitriu, B. Iker, K. Malik, H. Leung, W. W. Mohn, G. G. Hillebrand, *MBio* **2019**, *10*, e00839.
[6] A. Bonifaz, R. Rojas, A. Tirado-Sánchez, D. Chávez-López, C. Mena, L. Calderón, P. O. María, *Mycopathologia* **2016**, *181*, 671.
[7] R. Adam, *Pediatr Dermatol* **2008**, *25*, 427.
[8] D. Ward, A. Fleischer, S. Feldman, D. Krowchuk, *Arch Peds Adol Med* **2000**, *154*, 943.
[9] W. E. Jordan, K. D. Lawson, R. W. Berg, J. J. Franxman, A. M. Marrer, *Pediatr Dermatol* **1986**, *3*, 198.
[10] S. Adalat, D. Wall, H. Goodyear, *Pediatr Dermatol* **2007**, *5*, 483.
[11] U. Blume-Peytavi, V. Kanti, *Pediatr Dermatol* **2018**, *35*, 19.
[12] P. H. Andersen, A. P. Bucher, I. Saeed, P. C. Lee, J. A. Davis, H. I. Maibach, *Contact Dermatitis* **1994**, *30*, 152.
[13] K. W. Buckingham, R. W. Berg, *Pediatr Dermatol* **1986**, *3*, 107.
[14] R. W. Berg, K. W. Buckingham, R. L. Stewart, *Pediatr Dermatol* **1986**, *3*, 102.
[15] R. W. Berg, *Adv Dermatol* **1988**, *3*, 75.
[16] R. W. Berg, *Pediatrician* **1987**, *14*, s27.
[17] R. Folster-Holst, *Pediatr Dermatol* **2018**, *35*, s10.
[18] A. N. Carr, T. DeWitt, M. J. Cork, L. F. Eichenfield, R. Fölster-Holst, D. Hohl, A. T. Lane, A. Paller, L. Pickering, A. Taieb, T. Y. Cui, *Pediatr Dermatol* **2019**, *37*, 130. https://doi.org/10.1111/pde.14047
[19] M. G. Kalra, K. E. Higgins, B. S. Kinney, *Am Fam Physician* **2014**, *89*, 569.
[20] Y. Tüzün, R. Wolf, B. Engin, A. S. Keçici, Z. Kutlubay, *Clin Dermatol* **2015**, *33*, 420.
[21] F. Akin, M. Spraker, R. Aly, J. Leyden, W. Raynor, W. Landin, *Pediatr Dermatol* **2001**, *18*, 282.
[22] R. W. Berg, *Pediatrician* **1987**, *14*, 27.
[23] C. E. Shannon, *Bell System Tech J* **1948**, *27*, 379.
[24] W. H. Kruskal, W. A. Wallis, *J Am Stat Ass* **1952**, *47*, 583.
[25] J. Reizer, A. Reizer, M. H. Saier Jr, *Microbiol Res* **1990**, *141*, 1061.
[26] M. K. Bacic, C. J. Smith, *Curr Protoc Microbiol* **2008**, *9*, 13C.
[27] Y. Zheng, Q. Wang, L. Ma, Y. Chen, Y. Gao, G. Zhang, S. Cui, H. Liang, L. Song, C. He, *Exp Dermatol* **2019**, *28*, 1289.
[28] N. N. Schommer, R. L. Gallo, *Trends Microbiol* **2013**, *21*, 660.
[29] D. W. Mackenzie, *Appl Environ Microbiol* **1971**, *22*, 678.
[30] R. D. King, J. C. Lee, A. L. Morris, *Infect Immun* **1980**, *27*, 667.
[31] I. R. Makhoul, I. Kassis, N. Hashman, P. Sujov, *Pediatrics* **2001**, *108*, 16.
[32] E. C. Evans, M. Gray, *JWOCN* **2003**, *30*, 11.
[33] G. L. Darmstadt, J. G. Dinulos, *Pediatr Clin* **2000**, *47*, 757.
[34] S. Adalat, D. Wall, H. Goodyear, *Pediatr dermatol.* **2007**, *5*, 483.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Figure S1. Unweighted Unifrac Analysis for Bacterial Population by Site and Rash Severity

Table S1. Swab Sample Count per Site and Rash Grade

Table S2. Microbiome 16s Sample Information and Reads Counts

Table S3. Mycobiome ITS Sample Information and Reads Counts

How to cite this article: Teufel A, Howard B, Hu P, Carr AN. Characterization of the microbiome in the infant diapered area: Insights from healthy and damaged skin. *Exp Dermatol* 2021;30:1409–1417. https://doi.org/10.1111/exd.14198