Skin Colonization by *Malassezia* Species in Neonates

**A Prospective Study and Relationship With Neonatal Cephalic Pustulosis**

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**Objectives:** To assess skin colonization by *Malassezia* species in full-term healthy newborns, to investigate factors associated with colonization, and to look at acne-like cephalic pustulosis associated with this carriage.

**Design:** Samples were obtained from neonates and their mothers 0 to 5 days after birth and again 3 weeks later. Clinical patterns of common acnelike pustulosis were reported as mild (<10 papulopustules), moderate (≥10 papulopustules), or absent. Direct examination and culture of sample. Identification of yeasts was based on microscopic and physiologic criteria.

**Setting:** A maternity hospital and the pediatric dermatology unit of a university hospital.

**Participants:** Consecutive series of 102 neonates and their mothers.

**Main Outcome Measures:** Incidence of skin colonization and type of *Malassezia* species found in neonates and correlation with neonatal cephalic pustulosis (neonatal acne).

**Results:** At the first visit, 11 neonates and 36 mothers had cultures positive for *Malassezia*. *Malassezia sympodialis* and *Malassezia globosa* were preferentially cultured. At 3 weeks, 29 (52%) of 56 neonates and 18 (32%) of 56 mothers had cultures positive for only *M sympodialis* and *M globosa*. Breastfeeding was not associated with a higher prevalence of *Malassezia* carriage in neonates. *Malassezia* colonization was higher when pustulosis was more severe and *M sympodialis* was found in pustules.

**Conclusions:** *Malassezia* colonization begins at birth and increases in the first weeks of life. A high prevalence of *M sympodialis* in neonates is noted from birth. Its association with neonatal acne is confirmed. Further investigation is needed to study the role of sebum secretion rate and quality in the neonatal period.

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**NEWBORN SKIN is essentially sterile at birth.** Ex-cept for the transient flora originating mostly from the mother's genital tract, which can be potentially life-threatening for the newborn,2 bacteria of the newborn's resident flora are not cultured at birth but appear within the first hours of life. By age 6 weeks, the total number of organisms is comparable to that found in adults. *Staphylococcus epidermidis* is the most common bacterium.3 In preterm infants, staphylococci colonize skin earlier than *Propionibacterium* and *Malassezia* species, which have a slower growth rate and might require greater maturation of epidermal structures.1 Yeast flora4 is represented by nonlipophilic yeasts (*Candida* species) and lipophilic yeasts (*Malassezia* species). *Candida albicans* is not a regular member of the cutaneous microflora.5,6 Conversely, *Malassezia* organisms are saprophytic of normal human adult and child skin.5,7 Although it can be implicated in some systemic neonatal infections,7 *Malassezia* species in healthy neonates are associated with the common acnelike pustulosis of the cephalic area.10-15 Recent identification and differentiation of *Malassezia* species have opened new avenues for investigations.16-18

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This study aimed to assess skin colonization by *Malassezia* species in full-term healthy newborns and to investigate the factors associated with colonization. Another aim was to assess possible skin manifestations associated with this carriage, especially neonatal acnelike cephalic pustulosis.

**RESULTS**

The overall results are given in **Table 1**.

**FIRST VISIT**

While on the maternity unit, samples were obtained from 102 neonates and their mothers 0 to 5 days after birth. All the new-
PARTICIPANTS AND METHODS

FIRST VISIT

Samples were first obtained from neonates and their mothers on the maternity unit of the Pellegrin University Hospital 0 to 5 days after birth (December 1, 1997, to February 28, 1998). The mothers gave informed consent and agreed to return for a follow-up outpatient visit at the pediatric dermatology unit 3 weeks later. A complete skin examination of the newborns was performed. Type of feeding, breast or formula, was recorded. Children were defined as formula fed when they had never received any breastfeeding. Antimicrobial drug use (topical antiseptic or antibiotic agents and oral antibiotics) was recorded. The ethnic origins of the mother and father were noted. Social class was defined arbitrarily by the mother's occupation at the time of the child's birth according to the 1950 British classification of the Registrar.19

SECOND VISIT

Neonates and their mothers attended the outpatient visit for a second sampling at a mean±SD age of 21±5 days. Information about skin care was noted, and a complete skin examination was performed. Clinical patterns of common acnelike pustulosis were reported as mild (<10 papulopustules), moderate (≥10 papulopustules), or absent. Pustular material was obtained when technically possible.

PROCEDURES AND LABORATORY INVESTIGATIONS

Each sample from newborns was obtained by the same operator (V.B.) using a skin swab applied on a 1-cm² area of the cheek. Sampling of the mothers was performed on the neck using identical techniques. Whenever feasible, newborns were obtained from pustules using a microlance after disinfection with 0.1% alcoholic hexamidine di-isethionate solution. Technical limitations to sampling were the size of the lesion and the anxiety of the mothers. Pustular material was directly applied to a glass slide for direct examination after May-Grünwald-Giemsa staining. Skin swabs and pustule smears were then seeded in modified Dixon agar medium (3.6% malt extract, 0.6% peptone, 2.0% dextrose, 0.2% glycerol, 0.2% oleic acid, 0.05% chloramphenicol, 0.05% cycloheximide, and 1.5% agar [pH 6]). Colonies were counted after 15 days of incubation at 32°C. Identification of yeasts was based on microscopic (after May-Grünwald-Giemsa staining) and physiologic criteria, namely, Tweens and cremophor EL assimilation test, with splitting of esculin according to Guillot et al16 and Mayser et al.17

STATISTICAL ANALYSIS

Comparison of proportions was performed using the Fisher exact test. A test for trend in proportions was used to compare Malassezia-positive participants according to clinical patterns.

Table 1. Overall Clinical and Mycological Data

<table>
<thead>
<tr>
<th>Subject</th>
<th>Malassezia sympodialis</th>
<th>Malassezia globosa</th>
<th>M sympodialis + M globosa</th>
<th>Malassezia slooffiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonates</td>
<td>Girls 6 0 1 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boys 4 0 0 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subtotal</td>
<td>10 (91)</td>
<td>0</td>
<td>1 (9)</td>
<td>0</td>
</tr>
<tr>
<td>Mothers</td>
<td>15 (42)</td>
<td>16 (44)</td>
<td>4 (11)</td>
<td>1 (3)</td>
</tr>
</tbody>
</table>

Table 2. Initial Mycological Data in 11 Neonates and 36 Mothers With Positive Culture Findings

borns were born at term and were in good health. Routine skin care of the face was done with isotonic sodium chloride solution. No newborn had received antimicrobial local treatment or oral antibiotics, except eyedrops to prevent gonococcal ophthalmia.

The initial mycological data in neonates and mothers are reported in Table 2. Eleven percent of neonates and 36% of mothers had cultures positive for Malassezia organisms. Malassezia sympodialis and Malassezia globosa were preferentially cultured. One mother's culture was positive for Malassezia slooffiae.

Of 10 M sympodialis-positive neonates, 6 had M sympodialis-positive mothers, 1 had a M globosa-positive mother, and 3 had Malassezia species-negative mothers. The mother of the neonate positive for M sympodialis and M globosa was also Malassezia negative. Sixty-seven percent of Malassezia-negative newborns had Malassezia-negative mothers.

FOLLOW-UP VISIT

A total of 56 mothers and their newborns attended the outpatient visit at a mean±SD of 21±5 days (Table 3). There was no significant difference in the mothers' mycological status at birth between the group that completed the study and the group that did not (data not shown). Twenty-nine neonates (52%) and 18 mothers (32%) had positive cultures that grew only M sympodialis and M globosa. Of 25 M sympodialis-positive neonates, 7 (28%) had the same species as their mother; 4 (16%) had mothers positive for M sympodialis and M globosa. The 3 M globosa-positive neonates and the neonate positive for M sympodialis and M globosa showed no association with their mother's status (2 newborns) or a partial association (2 newborns). Sixty-three percent of
Malassezia-negative neonates had Malassezia species-negative mothers.

Breastfeeding (49 newborns) was not associated with a higher prevalence of Malassezia carriage in neonates (n = 23) (Fisher exact test, \(P = .11\)). However, the formula-only group was small (n = 7). No difference in Malassezia colonization was found according to social class (data not shown). White individuals were overrepresented (n = 48, 86%), and we could not check whether ethnic origin of the mother contributed to the type of colonization. The use of cosmetic products for routine skin care of the face did not interfere with Malassezia colonization (data not shown).

### CEPHALIC PUSTULOSIS

In affected patients, the pustulosis developed between day 5 and 3 weeks. When noted, lesions consisted of red papules of pinpoint size (first stage), papulopustules (second stage), or overt pustules (third stage), mostly located on the cheeks, chin, and forehead. Extension could occasionally be found on other seborrheic areas, such as the scalp or upper neck. No comedos were observed. Mycologic data at 3 weeks were compared with clinical patterns of neonatal cephalic pustulosis (Table 4). Thirty-seven (66%) of 56 newborns had lesions classified as mild or moderate. Eleven pustules were sampled successfully, and 4 were positive on direct examination and culture: 3 cultures grew _M sympodialis_ and 1 grew _M globosa_. Two of these 11 pustules had positive findings on direct examination and negative culture results, and 2 of 11 had positive culture findings and negative findings on direct examination. Of 11 children with pustules sampled, 3 had negative skin culture findings. Of the 4 patients with positive pustules (by direct examination and culture), all had positive skin culture results, and 3 were highly colonized (50-500 colonies). There was a correlation between positive culture findings using skin swabs on cheeks and clinical severity of presentation. Three of 11 children with successfully sampled pustules at 3 weeks already had colonization at day 5. Of these, 2 had positive pustules at 3 weeks (by direct examination and culture).

### Table 3. Mycological Data at 21 ± 5 Days in 29 Neonates and 18 Mothers With Positive Culture Findings

<table>
<thead>
<tr>
<th>Subject</th>
<th>Malassezia sympodialis</th>
<th>Malassezia globosa</th>
<th><em>M sympodialis</em> + <em>M globosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>12</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Boys</td>
<td>13</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Subtotal</td>
<td>25 (86)</td>
<td>3 (10)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Mothers</td>
<td>14 (78)</td>
<td>2 (11)</td>
<td>2 (11)</td>
</tr>
</tbody>
</table>

### Table 4. Mycological Data at 3 Weeks and Correlation With Severity of Neonatal Cephalic Pustulosis in 56 Neonates*

<table>
<thead>
<tr>
<th>Malassezia Culture</th>
<th>Moderate (n = 9)</th>
<th>Mild (n = 28)</th>
<th>Absent (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>7 (78)</td>
<td>16 (57)</td>
<td>6 (32)</td>
</tr>
<tr>
<td>Negative</td>
<td>2 (22)</td>
<td>12 (43)</td>
<td>13 (68)</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) of patients. \(P = .02\), test for trend in proportions.

Balanced distribution of the 2 species (*M sympodialis* and _M globosa_). Differences in sebum secretion rate and sebum composition could probably explain some differential growth patterns of each yeast; particularly, the endocrine environment of pregnancy could be more suitable for the growth of _M globosa_ since, as at 3 weeks, the relative percentage of _Malassezia_ species was nearly the same as in children.

_Malassezia_ is found in more than 90% of adults\(^{21}\); in children, this yeast has been found in 50% of healthy newborns at birth and in 80% after 7 days of life\(^{6}\) or, in a mostly premature population, from 13% in the first day of life to 77% between 1 and 3 month of age.\(^{7}\) The following differences in methods should be kept in mind if comparisons with our data are envisaged. Previous studies were performed before the taxonomic revision that has introduced identification and differentiation of the new _Malassezia_ species, based in our study on culture conditions and morphologic and physiologic criteria. Sampling the cheeks on a 1-cm\(^2\) area permitted standardization of the method, and this site correlated better with the usual location of neonatal cephalic pustulosis. The scalp and chest were preferentially sampled in another study.\(^{7}\) Finally, participant recruitment on the maternity unit could assess skin colonization of the full-term healthy newborn.

Progressive colonization by Malassezia species from birth was recently proposed by Eastick et al.\(^{15}\) and Niamba et al.,\(^{18}\) but infants in these studies were recruited in a neonatal care unit.

Our data prove that colonization by _Malassezia_ species begins in the first days of life (11% at day 5) and increases during the first weeks (52% at day 21). Maternal _Malassezia_ colonization was stable at the 2 visits in contrast to _Malassezia_ specificity. The rate of carriage at 3 weeks was higher in newborns than in mothers (52%}

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Yeasts of the genus _Malassezia_ are components of the microflora of human skin and of many warm-blooded animals.\(^{4,20}\) However, researchers do not agree on the age at which skin is colonized after birth.\(^{3,6,7,21}\) Recently, the taxonomic revision of the lipophilic yeast genus using morphologic, ultrastructural, physiologic, and molecular biological methods has included 7 species comprising 3 former taxa ( _Malassezia furfur_, _Malassezia pachydermatis_, and _M sympodialis_) and 4 new taxa ( _M globosa_, _Malassezia obtusa_, _Malassezia restricta_, and _M slooffiae_).\(^{16}\)

In this study, based on samples obtained from mothers, _M sympodialis_ and _M globosa_ were the exclusive residents of female skin, except for 1 isolate of _M slooffiae_. In children, _M sympodialis_ was the most prevalent _Malassezia_ species in the first 3 weeks of life. On the maternity unit, _Malassezia_ species-positive mothers had a more balanced distribution of the 2 species (_M sympodialis_ and _M globosa_). Differences in sebum secretion rate and sebum composition could probably explain some differential growth patterns of each yeast; particularly, the endocrine environment of pregnancy could be more suitable for the growth of _M globosa_ since, as at 3 weeks, the relative percentage of _Malassezia_ species was nearly the same as in children.

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Our data prove that colonization by _Malassezia_ species begins in the first days of life (11% at day 5) and increases during the first weeks (52% at day 21). Maternal _Malassezia_ colonization was stable at the 2 visits in contrast to _Malassezia_ specificity. The rate of carriage at 3 weeks was higher in newborns than in mothers (52%
vs 32%), but the sampling site was different. The elevated flow of sebum in neonates owing to sebaceous gland hyperactivity could also partially explain this finding.

The mother seems to be the first reservoir for the child’s colonization. At birth, 60% of M. sympodialis–positive neonates shared the same yeast species with their mother, as well as 44% at 3 weeks. A two-thirds correlation was also obtained in the Malassezia species-negative pairs at birth and at 3 weeks. A comparative molecular approach using restriction fragment length polymorphisms of yeast isolates from mothers and children could be interesting to validate this finding. To explain an absence of complete correlation, it remains possible that Malassezia species are not equally distributed on the skin of the mother and that M. sympodialis is more adapted to the skin of newborns than is M. globosa. Alternatively, other sources such as nursery personnel or other family members may be important to consider in future studies.

If maternal factors are implicated in Malassezia colonization at birth, close contact with the mother during breastfeeding and social class origin do not seem to affect the composition of the newborn’s skin flora. Furthermore, variations in routine skin care did not significantly affect skin colonization. The child’s intrinsic characteristics should probably be considered more closely, especially concerning sebum production.

In adults, tinea versicolor, seborrheic dermatitis, and folliculitis are well-known dermatoses in which Malassezia organism is implicated as an etiologic agent. In neonates, the possible role of Malassezia species in the cause of facial acniform pustulosis was first suggested by Aractingi et al and subsequently by other authors. More specifically, M. sympodialis has been proposed to be a triggering agent for this transient rash. Our data suggest that Malassezia colonization is higher when the pustulosis is more severe (Table 3). Moreover, M. sympodialis grew in 3 cultures from 4 pustular samplings. These results confirm, in nonselected neonates, that M. sympodialis plays a role in common neonatal cephalic pustulosis. However, cephalic pustulosis with negative mycological data suggests multifactorial causes for this common disorder.

In conclusion, Malassezia colonization of the skin begins at birth and increases within the first weeks of life. A high prevalence of M. sympodialis in neonates is noted from birth. Its association with neonatal acne is confirmed. Environmental factors and maternal contact probably affect this colonization, but neonatal skin characteristics are probably important. Further investigation is needed to study the role of sebum secretion rate and quality in the neonatal period. Other studies should also address the role of culture medium specificity for the cultivation of each species.