

Research Article

## A study on the extent, aetiology and associated factors of dandruff in a group of medical students and the in vitro effects of antidandruff preparations

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### Abstract

**Introduction:** Dandruff is a scalp disorder, characterized by itching and rapid skin flaking of the scalp of a person. Yeasts of the genus *Malassezia* are responsible for dandruff. The prevalence of dandruff in South Asia is 60.1%, whereas in the world it is 50%. The objectives of this study were to ascertain the prevalence, describe associated factors, isolate *Malassezia sp.* from dandruff samples of medical students and test the anti-fungal effects of commercially available shampoo and crude extracts of commonly used herbs against *Malassezia*.

**Methodology:** Ninety five students were chosen by systematic sampling. A self-administered questionnaire was used for data collection. Samples of skin flakes/swabs was collected from the scalp of participants. Specimens were examined using three direct microscopic methods. Fungal colonies isolated on Sabourauds/Dixon Agar were identified using Gram stain and catalase test. Antifungal activity of six shampoo brands and crude extracts of eleven herbs were tested against isolated *Malassezia sp.* by agar well/disc diffusion method.

**Results:** Prevalence of dandruff was 64.2% in the study group. The typical 'spaghetti and meatball' appearance was seen in the skin flakes taken from 44 of 61 (72.1%) students. *Malassezia sp.* was isolated only from 49 of the 61 students (80.3%). In-vitro, anti-malassezian effect (Inhibition zone (IZ)>10mm) was observed in 4 of the 7 tested 'anti dandruff' shampoos. Crude extracts of fresh leaves of *Psidium guavava* (guava), flowers of *Punica granatum* (pomegranate) and fruits of *Solanum lycopersicum* (tomato) and *Citrus hystrix* (Kaffir lime) showed IZs > 10mm.

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**Conclusion:** Prevalence of dandruff in the studied group of medical students was higher than in the Asian population. Four of the tested ‘anti dandruff’ shampoos and crude extracts of leaves of *P. guavava* (guava), fruits of *S. lycopersicum* (tomato), *C. hystrix* (kaffir lime), and flowers of *P. grantum* (pomegranate) showed an in-vitro inhibitory effect on the growth of *Malassezia* sp.

*Keywords:* dandruff, *Malassezia* sp., anti-fungal effects, herbals

## Introduction

Dandruff is described as a scalp disorder which is characterized by itching and abnormal and rapid turnover of the outermost layer of the skin of the scalp of a person.<sup>1</sup> The dead skin is released usually as patches and flakes of grayish white color.<sup>2</sup> Seborrheic dermatitis (SD) and dandruff are continuation of the same condition that affects the areas which have high sebum secretion like the face, upper chest and back.<sup>1</sup>

Clinical symptoms associated with dandruff are skin flaking, itching, irritation and the feeling of a dry or tight scalp. Loosely adherent, small white or gray flakes are characteristic of dandruff whereas SD presents a yellowish, oily scale.<sup>3</sup>

There are three main factors that aid the formation of dandruff. They are fungal infections of the scalp, sebaceous secretion and individual sensitivity to substances.<sup>4</sup>

The presence of excess sebum over the scalp, gender, age, region of the scalp and micro-organisms are known as the risk factors for dandruff formation.<sup>5</sup> The most widely accepted microbial organisms are the lipophilic yeasts which belong to the genus *Malassezia*. Frequent exposure to sunlight, over shampooing, frequent combing, use of some cosmetic products and exposure to dust and dirt can be identified as non – microbial causes of dandruff.<sup>6</sup>

Though not a disease with adverse clinical outcomes, dandruff could make the sufferer feel self-conscious and embarrassed. It is a known fact that dandruff affects self-esteem and confidence.<sup>7</sup> SD and dandruff affects half of the adult population due to intrinsic and environmental factors.<sup>1</sup> Some studies have shown that 46-50% of the population is affected with dandruff.<sup>8,9</sup> Dandruff was found to be more common among males than females. South Asia has reported a greater dandruff prevalence (60.1%) compared to other regions of the world (up to 50%) .<sup>8,9</sup>

There are no published data on the prevalence or etiology of dandruff in the Sri Lankan population. However, in the experience of the investigators, dandruff is a common problem among Sri Lankans, including university students. This study was therefore planned to assess the prevalence of dandruff among medical students, risk factors and the possibility of *Malassezia* sp. as a causative factor for dandruff. In addition, the in-vitro effectiveness of commercially available shampoos and crude extracts of commonly used herbs against *Malassezia* were tested against the isolated *Malassezia* sp.

## Methods

This study was conducted in the Department of Microbiology, Faculty of Medicine and Allied Sciences (FMAS), Rajarata University of Sri Lanka (RUSL), for 6 months from January 2019. Approval for the study was obtained from the Ethics Review Committee, FMAS, and RUSL (ERC/2018/39). Informed written consent was obtained from each participant at recruitment to the study.

The study was conducted in two sections.

**Section 1** was a cross sectional descriptive study aimed at assessing the prevalence of dandruff, and its associated factors in third year medical students of FMAS, RUSL and isolating *Malassezia* sp. from students identified as having dandruff.

The study population was the third year medical students of FMAS. The sample size was calculated using the formula,  $n = Np(1-p) / [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$ .<sup>10</sup>

The population size (p) was 190 (number of medical students in third academic year). Hypothesized percentage of dandruff was taken as 50% as there are no available data in Sri Lanka.<sup>9</sup> Confidence limit was taken as 10%. The calculated minimum sample size was 87 for 99% confidence interval. With 10% correction for the non-consenting participants, a minimum sample of 95 was targeted and students were recruited according to systematic random sampling technique and alternatively from their University index number.

A questionnaire based study, clinical examination of the scalp, specimen collection and processing were done according to the following methods.

### ***Questionnaire***

A self-administered questionnaire was given for the collection of data. The questionnaire included demographic data, assessment of symptoms of dandruff, availability of the risk factors, and the effects of dandruff on the participant's lives according to their point of view. The clinical severity of dandruff was estimated using Adherent Scalp Flaking Score (ASFS) on the scalp using a scale from 1 to 5 depending on the appearance of skin flakes. The investigators were trained on the use of ASFS by the supervisor under the guidance of the dermatology unit. The scores on 8 zones (right frontal, left frontal, right parietal/temporal, left parietal/temporal, right occipital and left occipital) of scalp were individually measured and recorded in a specially designed sketch of sub regions of the scalp, and the total score was recorded.<sup>9</sup> The severity of dandruff was categorized as follows, Mild dandruff when ASF score was 11-20, Moderate dandruff when the ASF score was 21-30 and severe dandruff when the ASF score was >30.<sup>9</sup>

### ***Clinical specimen collection and processing***

#### **Participants with visible skin flakes**

Easily detachable skin flakes were carefully collected from each participant from selected sites of the scalp using sterilized epilation forceps.<sup>2,11</sup> The specimen was then transferred onto dark sampling paper. Each sample was labelled with the participant's serial number and date of collection of the sample and transported to the laboratory for processing.

### **Participants without visible skin flakes**

Sterile cotton swabs which were autoclaved were rubbed onto the scalp surface (between the hairs) in a zigzag pattern, to cover a total surface of 4 cm<sup>2</sup> in a non-overlapping manner.<sup>11</sup> The swab was then placed in a sterile test tube and transferred to the laboratory for processing

### ***Specimen processing and analysis***

#### **Direct microscopy**

A drop of 10% potassium hydroxide, KOH with blue parker ink and 1% eosin were introduced onto a slide containing the sample and covered with a cover slip.<sup>11</sup> The sample was then heated over a Bunsen flame to remove bubbles. The slides were viewed using x10 and x40 objectives with closed iris diaphragm of the lens of the condenser.

#### **Culture**

Small amounts of the samples collected were introduced into Petri dishes containing Saboraud Dextrose Agar (SDA) and Dixon's agar using sterile forceps. The Petri dishes were labelled and incubated at 37 °C, up to a week.<sup>12,13</sup>

#### **Gram stain**

Gram stain was done on smears of the pure culture obtained after one weeks of incubation at 37 °C.

**Section 2** was the in vitro assessment of antifungal activity of commercially available shampoo and herbal extracts against *Malassezia* sp.

Using a sterile loop, 3 to 5 colonies taken from subcultures of isolated *Malassezia* sp. were mixed in 5 ml of distilled water and turbidity was adjusted to the 0.5 McFarland standard. The yeast cells were inoculated on to the agar surface using a sterile cotton swab. A well grown culture of an isolated *Malassezia* sp. on Dixon agar medium was used for testing antifungal activity. Both disc diffusion method and well diffusion methods were used.

Commercially available 2% ketoconazole (Jenzen Cillag ltd), was used as the control, in the well diffusion method and the same suspension soaked in filter paper discs (Whatman no.1, 15 mm diameter), was used as the control in the disc diffusion method.

#### ***Agar well diffusion method***

The agar plate surface was inoculated by spreading one µL of the turbidity adjusted inoculum over the entire agar surface using a sterile cotton swab. A well with a diameter of 10mm was punched aseptically using a sterile cork borer and 10 µL of each test compound\crude extract without dilution was added to the well. In addition, 10 µL of 2% ketoconazole and (10 µL distilled water) were placed in similar wells as positive and negative controls respectively. The agar plates were incubated at 37 °C for five days to test for the antifungal effect. The diameter of the zone of inhibition was measured across the centre. A diameter of >10mm was taken as a significant anti malassezial effect.

### ***Agar disc diffusion method***

Dixon's agar plates were inoculated by spreading *Malassezia* yeast cells over the entire agar surface using a sterile cotton swab. Then, filter paper discs (Whatman no.1, 15 mm diameter), containing the test compound at a 100% concentration, were placed on the agar surface. The Petri dishes were incubated at 37 °C for 48 hours. In addition, discs containing ketoconazole and distilled water were placed on the media as positive and negative controls respectively.

### ***Antifungal effects of shampoo***

The shampoos used in the study were popular brands in the Sri Lanka market (names not disclosed to avoid any commercial implication). The tested shampoo brands were categorized into 2 groups as shown in Table 1.

**Table 1: Categories and constituents of shampoos**

Anti-dandruff shampoo		Non 'anti dandruff' shampoo	
Brand A	Cetrimide	Brand 1	Sodium laureth sulphate, , Dimethicone, Glycol distearate
Brand B	Tea tree oil, Grape seed, Aloe	Brand 2	Bacopa (Lunuwila)
Brand C	Lunuwila extract , Fenugreek , Argan oil	Brand 3	Sodium laureth sulphate, Dimethiconol, Cocamidopropyl betaine
Brand D	Chlorhexidine gluconate 2%, Ketoconazole 1%		
Brand E	Godapara extract		
Brand F	Climbazole		
Brand G	Tea tree oil, Zinc Prythion		

The tested shampoo with the active ingredient and/or the herbal extracts contained in them according to the manufacturers' notes.

### ***Antifungal effect of herbs***

Herbs tested for anti-fungal effects in the study are shown in Table 2. The herbs were authenticated at the National herbarium, Royal Botanical Gardens, Peradeniya.

**Table 2: Herbs tested in the study**

Herbal part		Name of herb		
		English	Sinhala	Tamil
Leaves	<i>Azadirachta indica</i>	Neem	Kohomba	Veppai
	<i>Elaeocarpus serratus</i>	Ceylon olive	Veralu	Karamaram
	<i>Psidium guvava</i>	Guava	Pera	Caitiyam koyyā
	<i>Aloe vera</i>	Aloe	Komarika	Karrālai
	<i>Bacopa monnieri</i>	Water hyssop	Lunuwila	Nirppirami
	<i>Punica grantum</i>	Pomegranate	Delum	Madulai
Flowers	<i>Punica grantum</i>	Pomegranate	Delum	Madulai
Fresh fruits	<i>Dillenia retusa</i>	*	Godapara	Naiteku
	<i>Citrus aurantiifolia</i>	Lime	Dehi	Campas a
	<i>Solanum lycopersicum</i>	Tomato	Thakkali	Takkāai
	<i>Dillenia triquetra</i>	*	Diyapara	Naiteku
	<i>Citrus hystrix</i>	Kaffir lime	Kudalu dehi	Nardanga

\*No English names are available as these plants are endemic to Sri Lanka

The collection of herbs was done between 1.3. 2019 to 10.3. 2019. Fresh leaves, flowers and fresh fruits were washed using flowing water and air dried for 20 minutes to evaporate water drops. Ten grams of selected parts of each herb was weighed and then ground separately using a mortar and pestle. The pulps which were formed by each sample were squeezed in cheese cloths and the filtrate considered as a 100% extract of the plant.

## Results

### Section 1

Of the 95 students sampled during the period of the study, 61 (64.2%) had dandruff of whom 42 (68.9%) were females. Of the students with dandruff, 54 (88.5%) had mild dandruff and 7 (11.5%) moderate dandruff. Table 3 gives the percentage and frequency of students having dandruff of different grades of severity.

**Table 3: Severity of dandruff according to ASFS grading**

Clinical category	ASF Score	Participants	
		Number	%
Mild dandruff	1-10	21	34.4
	11-20	33	54.1
Moderate dandruff	21-30	7	11.5
Severe dandruff	>30	0	0

**Table 4: Significant values of associated risk factors of dandruff**

Associated factors	Number of participants with dandruff	Number of participants without dandruff	P value
Excessive sweating	10	13	0.37
Regular physical exercise	35	15	0.64
Shampoo use	47	33	0.35
Soap use	10	9	0.56
Frequency of bathing (per week)			0.97
• 1	2	0	
• 2	2	1	
• 3	14	7	
• 4	19	14	
• 5	6	3	
• 6	2	2	
• 7	11	12	
Bathing time			0.37
• Morning	3	2	
• Day time	8	5	
• Afternoon	34	26	
• Night	11	6	
Blow dryer use	18	5	0.21
Conditioner use	31	16	0.48
Leave on conditioner	3	8	0.98
Chemically changed	9	10	0.98
Chemically processing times			1.00
• Never	47	29	
• Once a week	1	0	
• Once a month	1	1	
• Once every 6 months	1	1	
• Once a year	4	4	
• Once ever	2	4	
Dye hair	2	2	0.02

### Microscopy

The ‘spaghetti and meatball’ appearance, typical for KOH smears of *Malassezia* sp. was seen in 44 of 61 (72.1%) skin flake samples (Figure 1a). Of the 61 samples, *Malassezia* sp. was isolated from 49 (80.3%). None of the skin swabs (0/34) were positive for *Malassezia* sp. Figure 1b shows a Gram stained smear of isolated *Malassezia* sp.



**Figure 1a: Spaghetti and meatball appearance in KOH preparation of dandruff**



**Figure 1b: Gram stained smear of Malassezia sp. isolated from dandruff**

## Section 2

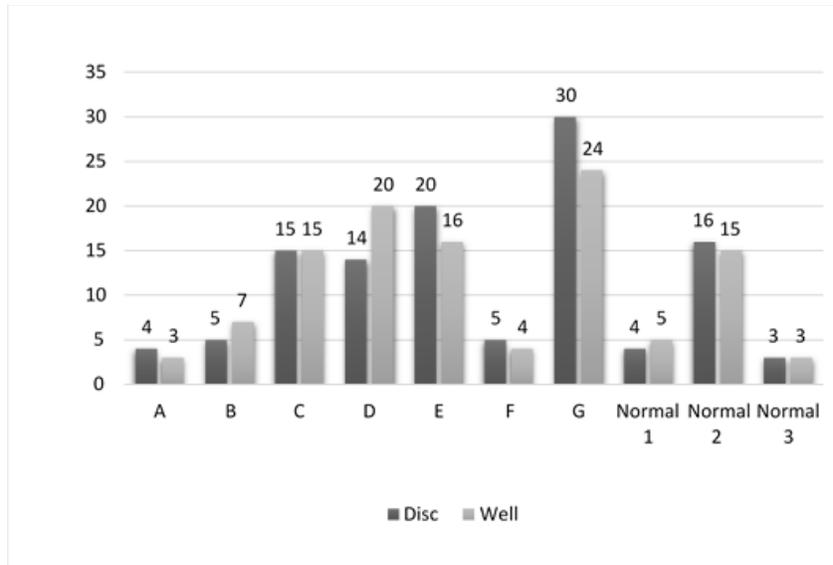
In-vitro, anti-malassezian effect (IZ >10mm) was observed in 4 of the 7 tested ‘anti dandruff’ shampoos. One of the three non anti dandruff shampoos showed anti-fungal effects against *Malassezia* sp. (Table 5). In-vitro, anti-malassezian effect (IZ >10mm) was demonstrated by 5 of the 11 tested crude extracts of herbs (Table 6).

**Table 5 : Inhibition zones of tested shampoo**

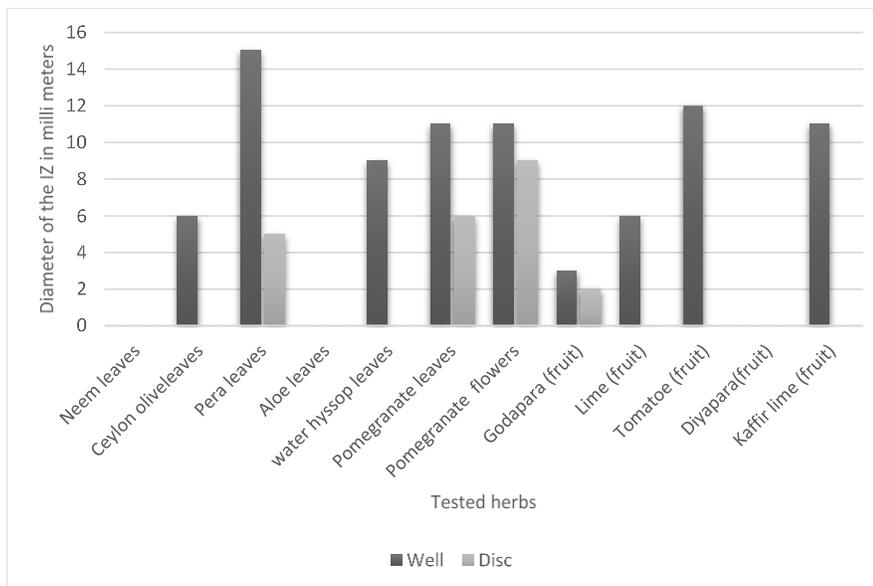
Tested shampoo	Active ingredients/herbal extract	IZ of test product (mm)		IZ of 2% ketoconazole (mm)	
		Well	Disc	Well	Disc
<b>Brand A</b>	Cetrimide	4	3	16	15
<b>Brand B</b>	Tea tree oil, Grape seed, Aloe	5	7	16	15
<b>Brand C</b>	Bacopa , Fenugreek , Argan oil	15	15	18	18
<b>Brand D</b>	Chlorhexidine, Ketoconazole 1%	14	20	18	18
<b>Brand E</b>	Dillenia retusa extract	20	16	16	17
<b>Brand F</b>	Climbazole	5	4	18	17
<b>Brand G</b>	Tea tree oil, Zinc Pyrithion	30	24	19	16
<b>Brand 1</b>	Sodium Laureth Sulphate, , Dimethicone, Glycol disterate	4	5	16	17
<b>Brand 2</b>	Bacopa	16	15	18	17
<b>Brand 3</b>	Sodium Laureth Sulphate, Dimethiconol, Cocamidopropyl Betaine	3	3	19	16

**Table 6: Inhibition zones of herbal preparations**

Tested herbs	Active ingredients/herbal extract	Preparation	IZ of test product (mm)		IZ of 2% Ketoconazole (mm)	
			Well	Disc	Well	Disc
<i>Azadirachta indica</i>	<i>Neem</i>	Crude extract of leaves	0	0	18	17
<i>Elaeocarpus serratus</i>	<i>Ceylon olive</i>	Crude extract of leaves	6	0	17	17
<i>Psidium guajava</i>	<i>Guava</i>	Crude extract of leaves	15	5	18	18
<i>Aloe vera</i>	<i>Aloe</i>	Crude extract of leaves	0	0	18	17
<i>Bacopa monnieri</i>	<i>Water hyssop</i>	Crude extract of leaves	9	0	19	18
<i>Punica grantum</i>	Pomegranate	Crude extract of leaves	11	6	17	16
<i>Punica grantum</i>	Pomegranate	Crude extract of flowers	11	9	16	16
<i>Dillenia retusa</i>	Godapara	Crude extract of fruits	3	2	18	16
<i>Citrus aurantiifolia</i>	Lime	Crude extract of fruits	6	0	19	17
<i>Solanum lycopersicum</i>	Tomato	Crude extract of fruits	12	0	17	17
<i>Dillenia triquetra</i>	Diyapara	Crude extract of fruits	0	0	18	16
<i>Citrus hystrix</i>	Kaffir lime	Crude extract of fruits	11	0	18	18



**Figure 2: Comparison of the inhibition zones shown by the tested shampoo**



**Figure 3: Comparison of the inhibition zones shown by the crude extracts of herbs**

## Discussion

The prevalence of dandruff in the tested population is 64.2%. Though previous studies are not available in Sri Lanka, one study done in the Asian region reported a prevalence of dandruff of 60.1%.<sup>8</sup> The symptoms of dandruff was similar to the present study. Dandruff is reported as being more prevalent among males than females. In a study done by Misery et al (2013) the prevalence among male participants was 20.7% compared to 12.8% in females.<sup>13</sup> In contrast, more females (63.9%) were found to have dandruff in the present study. The reasons for this difference need to be studied further. Only the use of hair dyes showed a statistically significant association with the presence of dandruff. Previous reports of an association between hair dye use and dandruff could not be found. The irritation of the scalp skin due to frequent use of hair dyes leading to increased susceptibility of skin for the growth of *Malassezia* sp. may explain this association

The organism was identified in the current study by its typical appearance in the KOH preparation and growth on Dixon agar medium, which is specific for yeasts of *Malassezia* genus. Species identification could not be carried out due to financial constraints.

Sample collection was done using the method described in Bhattacharyya et al.,(2017).<sup>10</sup> This method was selected because there is a possibility that *Malassezia* sp. could be present in normal scalp skin (as a member of the normal flora), even in the absence of dandruff.<sup>14</sup> However, in the present study *Malassezia* sp were not isolated from participants without dandruff who were therefore included as a control group (Table 4).

Both well diffusion method and disc diffusion methods were used in assessing antifungal activity. Though disc diffusion method is used in testing anti-bacterial effects of drugs, the well diffusion method is considered better for assessing the effects of aqueous preparations. Although both methods were used in the current study, the results of the well diffusion method were used in analysis as it is the recognized method for obtaining the inhibitory effects of aqueous preparations.

Various commercially available products as well as home remedies are used by people for treatment of dandruff. Some authors have referred dandruff as the most commercially exploited skin condition.<sup>13</sup> We have tested the anti malassezian effect of some commercially available preparations and commonly used herbal preparations. Interestingly, while some 'anti dandruff' shampoos (3/7) did not show in vitro anti malassezian effect, one 'non anti dandruff' shampoo showed significant anti malassezian activity. The current study assessed only the vitro activity of the tested compounds. The absence of in vitro anti malassezian activity in the advertised 'anti dandruff' shampoo is worthy of note. Three shampoo brands (Brand C, E, and brand 2) in which herbal extracts of 'Water hyssop' and 'Godapara' were stated as main ingredients, showed inhibition zones > 10mm. However, the crude extracts of the same herbs showed inhibition zones of only 9mm and 3mm respectively. Since the manufacturers did not state the method of extraction, the discrepancy cannot be explained at this stage. Further studies on the active ingredients in the herbs are required to determine the active compounds and most effective method(s) of extraction to maximize effectiveness of the products used for this purpose.

The current study assessed only the *in vitro* activity and did not study the clinical effectiveness of the tested products. Further studies are indicated to determine clinical effectiveness of commercially available shampoos as well as herbal remedies. In depth studies including clinical trials to test the efficacy and

safety of herbal preparations may lead to the discovery of new products with better ‘anti dandruff’ properties.

## Conclusion

Prevalence of dandruff in the studied group of medical students was higher than the Asian population. Four of the tested ‘Anti dandruff’ shampoos, crude extracts of leaves of Pera, fruits of Kudalu and Thakkali, and flowers of Delum showed in-vitro inhibitory effects (>10mm Inhibition Zones) on the growth of *Malassezia* sp.

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**Conflicts of Interests:** The authors declare that there are no conflicts of interests.

**Ethical statement:** Ethical clearance for the study was obtained from Ethics Review Committee, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka (ERC/2017/39)

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