Continuous increase of *Trichophyton tonsurans* as a cause of tinea capitis in the urban area of Paris, France: a 5-year-long study

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Abstract

Tinea capitis (TC) is a highly contagious fungal infection of the scalp due to dermatophytes in children. To obtain information on the epidemiology of TC in the urban area of Paris, we analysed the microbiological results of 3090 patients seen with suspected TC from October 2010 to September 2015 at Saint Louis hospital, Paris, France. A peak of TC was observed in 3–6 year-old children, followed by a progressive decrease until 16 years of age. Of the 1311 positive cultures, 95% (1246) yielded one of the three anthropophilic species [*Trichophyton tonsurans* (33.5%), *Trichophyton soudanense* (38.3%), or *Microsporum audouinii* (28.2%)]. When considering one TC case per family, we observed a significant increase of *T. tonsurans* (*P* = .018) during these 5 years. The increase was more pronounced (*P* = .0047) in patients of West-African descent (*n* = 666), and was at the expense of *M. audouinii* and *T. soudanense*. On the other hand, the Caribbean patients (*n* = 85) remained predominantly (72.9%) infected by *T. tonsurans*. Our results show a better virulence of *T. tonsurans* over other species as already reported. Since *T. tonsurans* has not been reported in Africa, the infection of patients of West-African descent probably took place in the Paris area by exchanges with Caribbean patients. This increase of TC due to *T. tonsurans* was observed in the context of griseofulvin being the only licensed paediatric treatment
for TC in France, which should deserve reappraisal because terbinafine may be more efficacious.

**Key words:** Tinea capitis, Trichophyton tonsurans, Trichophyton soudanense, Microsporum audouinii var. langeronii

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**Introduction**

Tinea capitis (TC), or scalp ringworm, remains a major public health concern in urban areas, where it is the most common superficial mycosis in children of school age. This fungal infection is caused by various dermatophytes that can be divided into three major groups according to the reservoir and the type of transmission: anthropophilic, zoophilic, or geophilic. TC results from infection by two main genera, *Microsporum* and *Trichophyton* spp., and many different species, including *Microsporum canis*, *Microsporum audouinii* var. *langeronii*, *Trichophyton tonsurans*, *Trichophyton soudanense*, and *Trichophyton violaceum*.

The epidemiology of TCs mainly depends on the geographical origin of the patients. In the mid-2000s, *M. canis* remained the main TC species in northern Europe, with mammal pets as the endemic reservoir, whereas anthropophilic *Trichophyton* species emerged in big metropolitan cities such as Madrid, Rotterdam, Stockholm, and London. In Paris, *T. soudanense* and *M. audouinii* were the main reported species, primarily due to the West-Africa origin of many patients, where these two anthropophilic species are endemic. However, *T. tonsurans* has been increasingly detected in Paris since 2000. Further, this species had already shown its ability to spread as it has also become the main species responsible for TC in the United States and in London since the 1990s.

We were therefore interested in knowing whether our epidemiology of TC has changed over the last 5 years and whether the increase of *T. tonsurans* already reported in our hospital was confirmed. Aside from the epidemiology of TC and the routes of transmission, knowledge of the species that causes the infection is important for therapeutic decision since all the species do not have the same susceptibility to antifungal drugs.

**Patients and methods**

**Design and data collection**

Saint-Louis Hospital is a 650-bed tertiary university hospital located in Paris, France, that is historically known for major clinical activity in dermatology. All suspected TC cases seen routinely in consultation at Saint-Louis Hospital were recorded from October 2010 to September 2015. Consultations for suspicion of dermatophytosis are open on weekdays with no appointment. Suspected lesions were sampled and demographic data (age, gender, geographical origin, familial links, and source of contact) were recorded on a specific form at each consultation as part of our usual procedure for standard diagnostics. According to the French Health Public Law (CSP Art L1121-1.1), such protocol does not require approval of an ethics committee and is exempted from specific informed consent application.

**TC definition and treatment**

A TC case was defined as displaying clinical signs such as alopecia, patchy hair loss, scaling, or scalp inflammation with positive direct microscopic examination and/or positive culture. The patients who presented no specific clinical signs and with noncontributive direct examination (negative or not performed because of lack of visible lesions) but had positive cultures were considered to be carriers. For patients without clinical lesions, sampling was performed by scraping or swabbing the scalp.

After hair and skin scrapings of suspected lesions were taken, a direct microscopic examination with Amman lactophenol (RAL Diagnostics, Martillac, France) was performed in the consultation room to confirm the lesion of the hair and to differentiate between fungal arthroconidia inside (indicative of endothrix *Trichophyton* sp.) or outside (indicative of ectothrix *Microsporum* sp.) the hair shaft. Afterward, the samples were inoculated on Sabouraud medium agar slants with gentamycin/clorachlamenicol with or without cycloheximide addition (Bio-Rad, Marnes la Coquette, France) and kept for 3 weeks at 26 °C with weekly checking. Species identification was based on macroscopic and microscopic characterization of the cultures.

When TC was diagnosed, the only treatment prescribed as the only one licensed in France for this indication was oral griseofulvin [breakable tablet of 250 or 500 mg; dose by body weight: <50 kg 20 mg/kg per day (single or divided dose) for 6–8 weeks; >50 kg 1 g per day (single or divided dose) for 6–8 weeks] to be taken during a meal. Imidazole or ciclopiroxolamine cream or lotion once a day were recommended for the first 4 weeks. Additional measures were recommended (cleaning of hairdressing tools, cap and scarf washing, untangling of braid hair). The same...
treatment and recommendations were given to carriers. All the patients were asked for a control visit after treatment completion. Cure was defined as a reduction or disappearance of clinical signs, no hair parasitism, and negative culture, whereas failure was defined as a persistence of clinical signs or carriage.16

Sequence analysis of ribosomal DNA internal transcribed spacer region (ITS)

Internal transcribed spacer region (ITS) sequencing was performed on 12 isolates of 7 species (2 T. tonsurans, 3 T. soudanense, 3 T. rubrum, 1 T. violaceum, 1 M. audouinii, 1 M. canis, and 1 Microsporum fulvum) to verify the phenotypic identification.2 DNA was extracted from 3-week old colonies using a MagNA Pure LC (Roche) with the large volume kit. All the PCR reactions were performed using a GeneAmp PCR System 9700 Thermocycler (Applied Biosystems) using two ITS primer pairs (ITS1-F: 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS2-R: 5′-GTCGGCTTCTTCCATCGATGC-3′; and ITS3-F: 5′-GATCGATGAAGAACGCAGC-3′ and ITS4-R: 5′-TCCCTCGTTATATGATATGG-3′)19 according to standard protocols of sequencing.20 The resulting chromatograms were corrected and assembled using BioEdit Sequence Alignment Editor (version 7.0.9.0) software (Ibis Biosciences, Carlsbad, CA, USA), and these corrected sequences were identified by using a BLAST search in the MycoBank database (http://www.mycobank.org/).

Statistical analyses

Qualitative variables were described as numbers (percentages). Quantitative variables were described as medians and interquartile ranges or means +/- standard deviations according to normality of distributions. The χ² tests and Wilcoxon nonparametric tests were used for appropriate comparisons. Trends in the incidence or recovery of the different species were tested by fitting to a linear model. The deviation from zero of the different slopes of the linear regression models was tested. All P values were two-sided with an assumed significance level of 5%. Statistical analyses and graphs were performed with Prism software v.6.0 (Graphpad).

Results

During this 5-year period, 3090 patients (male/female ratio 0.93, median age 8 years, interquartile range 4–28 years, range: 1 month–89 years) were examined for suspicion of TC. The majority of patients (1895; 61%) were sent by dermatologists of the hospital, 761 (25%) were sent by general practitioners or private practice dermatologists, 229 (7%) were sent by school medical doctors, 88 (3%) by the pediatric hospital associated with our hospital, 106 (4%) by other hospitals, and the information was unavailable for the remaining 11 (0.4%) patients. The majority of the patient population, 61% (2040 out of 3090), consisted of children under 16 years of age, with slightly more boys (53%, 1075 out of 2040). For the patient population above 16 years of age, there were slightly more female patients (61%, 638 out of 1050).

Taking into consideration only the first visit, that is, leaving out any subsequent consultations, we observed 1115 patients (36%) with TC and 226 patients (7.3%) as TC carriers among the 3090 patients tested without significant modification (P > .05) over the 5-year study (Table 1). The mean number of patients per year was 618 +/- 71 with 223 +/- 21 having TC and 45 +/- 13 being carriers.

Analysis of the cases of TC patients and carriers based on age and sex showed a peak of infection in 3 to 6-year-old children, followed by a progressive decrease until the age of 16 years (Fig. 1). The boys had more cases of TC than the girls (60.8%, 654 out of 1075 compared to 40.9%, 395 out of 965; P < .001), but there was a similar rate of carriers (7.3%, 79 out of 1075 compared to 8.3%, 80 out of 965; P > .05). For the adults (>16 years), there was no statistical difference in the rate of infection between women (8%, 51 out of 638) and men (3.6%, 15 out of 412; P = .23), but women were statistically more often carriers (8.8%, 56 out of 638) than men (2.7%, 11 out of 412; P = .0003).

We obtained 3372 hair samples from the 3090 patients. When restricting the culture analysis to the first positive sample for a given patient, we obtained 1311 positive cultures from 1085 cases of TC (30 of the 1115 microscopy-positive samples were culture-negative) and from 226 carriers. Agreement between microscopy and culture results was high (>99.9%). Six samples out of 1085 were misclassified by microscopic examination between endothrix and ectothrix. Of the 302 Microsporum spp. positive samples, five were classified as endothrix by microscopy, and of the 783 Trichophyton spp. positive samples, one was classified as ectothrix by microscopy. ITS sequencing of representative isolates of each species confirmed the phenotypic identification. As expected based on recent taxonomic studies,2 T. soudanense isolates were identified as T. rubrum. However, the name T. soudanense was used, although it is accepted as a synonym of T. rubrum,2 because T. soudanense is easily distinguishable based on phenotypic features on Sabouraud medium.18 Overall, the 1311 positive cultures from TC and carriers, yielded 476 T. soudanense, 418 T. tonsurans, 352 M. audouinii, 39 M. canis, 13 T. violaceum
Table 1. Distribution of the 3090 patients according to the clinical and microbiological observations and according to the quarters (Q1: January–March; Q2: April–June; Q3: July–September; Q4 October–December) of the 5 years of the study (October 2010 to September 2015).

| Quarter          | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | P value |
|------------------|--------|--------|--------|--------|--------|---------|
| Total patients (n = 3090) | 167    | 192    | 161    | 131    | 193    | 185     |
| Tinea capitis (n = 1115; % of n) | 60     | 64     | 56     | 34     | 50     | 69      |
| Trichophyton soudanense n = 406 (% of tinea capitis) | 30     | 11     | 21     | 22     | 21     | 28      |
| Microsporum audouinii n = 266 (% of tinea capitis) | 27     | 14     | 41     | 11     | 7      | 12      |
| Trichophyton tonsurans n = 360 (% of tinea capitis) | 12     | 22     | 16     | 8      | 16     | 21      |
| Microsporum canis n = 34 (% of tinea capitis) | 3      | 1      | 3      | 0      | 1      | 2       |
| Trichophyton violaceum n = 11 (% of tinea capitis) | 1      | 1      | 2      | 0      | 0      | 0       |
| other speciesa | 0      | 0      | 0      | 0      | 0      | 0       |
| Hair parasitism with negative culture n = 30 (% of tinea capitis) | 1      | 2      | 0      | 0      | 2      | 3       |
| Carriers (n = 226; % of n) | 10     | 18     | 8      | 5      | 11     | 16      |
| Microsporum soudanense n = 70 (% of carriers) | 3      | 5      | 1      | 3      | 5      | 4       |
| Microsporum audouinii n = 86 (% of carriers) | 5      | 12     | 10     | 6      | 0      | 5       |
| Trichophyton tonsurans n = 58 (% of carriers) | 2      | 3      | 3      | 1      | 4      | 7       |
| Microsporum canis n = 5 (% of carriers) | 0      | 0      | 0      | 0      | 0      | 0       |
| Trichophyton violaceum n = 2 (% of carriers) | 0      | 0      | 0      | 0      | 0      | 0       |
| other speciesb | 0      | 0      | 0      | 0      | 0      | 0       |
| Negatives (n = 1749; % of n) | 97     | 108    | 87     | 68     | 76     | 113     |

a Included: Trichophyton mentagrophytes (n = 3); Microsporum gypseum (n = 1); and Microsporum sp. (n = 1).
b Included: Trichophyton rubrum (n = 3); Trichophyton mentagrophytes (n = 2).
isolates, and 13 cultures yielded other species isolates (Table 1). Of note, three families of two individuals each were clustered around a pet animal and were infected with *M. canis*. The three major anthropophilic species (*T. soudanense*, *T. tonsurans*, and *M. audouinii*) represented 95% (1246 out of 1311) of the TC cases, with more patients found to be carriers of *M. audouinii* (24.4%, 86 out of 352) than of the two *Trichophyton* species (14.3%, 128 out of 894; *P* < 10^−4). Given the huge predominance of school age children, we looked for any difference between quarters for these three major species, knowing that school begins mid-September. We observed a slight increase in the first two quarters, from January to March (44.2%, 396 out of 500) and April to June (46.0%, 426 out of 500), compared to the third and fourth quarters from July to September (42.5%, 225 out of 305) and October to December (39.8%, 294 out of 444). However, this observation was without statistical significance (*P* > .05) (Table 1).

Due to their huge predominance, only the three major anthropophilic species were considered for subsequent analyses. Due to the study design, several issues were considered for the ensuing analysis. First, to avoid bias related to the size of outbreak, only one case was selected for each of the two outbreaks that occurred in day-care centres. One outbreak included three TC cases caused by *T. tonsurans* and no carriers. The other breakout had seven TC cases with *M. audouinii* and four carriers (Supplementary Table 1). Finally, to avoid distortion between quarters due to the low enrolment rate during summer months because of the summer break, we analysed the relative proportion of the different species rather than their absolute numbers. Overall, there were 880 culture positive TC cases that included 354 (40%) *T. soudanense*, 298 (34%) *T. tonsurans*, and 228 (26%) *M. audouinii*. We observed a statistically significant increase in the proportion of *T. tonsurans* over time (β = 0.678; *P* = .018) (Table 2). When restricting the analysis to culture positive TC cases from patients of West-Africa origin (n = 666), the trends in the increasing proportion of *T. tonsurans* was more pronounced (β = 0.914; *P* = .0047), whereas the incidences of *M. audouinii* and *T. soudanense* decreased (Fig. 2 and Table 2). In the TC positive cases in the Caribbean culture (n = 85), *T. tonsurans* remained predominant (n = 62; 73%), although *T. soudanense* was found at a low rate (n = 14; 16%).

Only 150 patients (123 TC and 27 carriers, i.e., 11% of the 1115 TCs and 12% of the 226 carriers) were sampled several times (mean 2; range 2–5) after treatment completion. Most of the cases of TC (n = 114) were initially due to one of three anthropophilic species (*M. audouinii*, n = 48; *T. soudanense*, n = 36; and *T. tonsurans*, n = 30).
Table 2. Repartition by quarter over the five-year study of the three major anthropophilic species responsible for tinea capitis (TC) according to the origin of the patients when considering only one case per family and one case per day-care epidemic (see supplementary Table 1).

| Quarter | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | P value |
|---------|--------|--------|--------|--------|--------|---------|
| All antropophilic TCs (n = 880) | | | | | | |
| T. soudanense (n = 354; 40.2%) (%) | 21 | 10 | 16 | 18 | 17 | .65 |
| M. audouinii (n = 228 ; 25.9 %) (%) | 12 | 18 | 11 | 3 | 10 | .08 |
| T. tonsurans (n = 298; 33.9%) (%) | 10 | 17 | 12 | 6 | 15 | .11 |
| Caribbean patients (n = 85; 9.6%) (% of n) | 28 | 26 | 24 | 17 | 31 | .02 |
| West Africa descent patients (n = 666; 75.7%) (% of n) | 25 | 10 | 4 | 1 | 10 | .36 |
| T. soudanense (n = 316; 47.4%) (%) | 17 | 6 | 15 | 14 | 16 | .5 |
| M. audouinii (n = 165; 24.8%) (%) | 7 | 10 | 4 | 1 | 10 | .11 |
| T. tonsurans (n = 185; 27.8%) (%) | 4 | 10 | 5 | 2 | 5 | .09 |
| T. soudanense (n = 14;16.5%) (%) | 0 | 1 | 0 | 0 | 0 | .98 |
| M. audouinii (n = 9; 10.6%) (%) | 0 | 0 | 0 | 0 | 0 | .28 |
| T. tonsurans (n = 62 ;72.9%) (%) | 0 | 3 | 2 | 1 | 8 | .36 |
For these three species, the first mycological control was performed with a median of 8 weeks (interquartile range: 6–14 weeks, range: 2–85 weeks) after the initial visit. We observed more incidents of failure for M. audouinii (58.3%; 20 persistent TC cases and 8 carriers) than for T. soudanense (32.8%; 12 persistent TC cases and 7 carriers) or T. tonsurans (26.7%; four persistent TC cases and four 4 carriers) (P = .02). Similarly, for the 27 carriers with a second consultation, 25 initially harbored one of the three anthropophilic species (12 M. audouinii, six T. soudanense, and seven T. tonsurans). At the second consultation, four patients remained carriers (one M. audouinii, one T. soudanense, and two T. tonsurans), and three patients evolved toward TC (two M. audouinii and one T. soudanense).

Discussion
This comprehensive retrospective analysis of cases of TC over 5 years of prospectively collected data showed the predominance of infection by the anthropophilic species T. tonsurans, T. soudanense, and M. audouinii. In addition, there were few cases caused by the anthropophilic Trichosporon violaceum and the zoophilic M. canis. This is in accordance with previous reports underlying the increase of these Trichophyton species in urban communities, in contrast to other European countries or other parts of France where Trichosporon violaceum or M. canis remain the predominant cause of TC. Since we do not know the number of people exposed, we cannot speak of prevalence but can instead discuss trends related to the samples processed in our laboratory, knowing that the total number of TC cases did not increase over the period of time studied, with a median of 221 cases per year (range 196–253). The global observation of the predominance of anthropophilic species confirmed what was already reported, with the main population concerned being 3 to 6 year-old boys. We analyzed our data according to quarters of the year, hypothesizing that some children could have been contaminated during vacation in Africa, followed by subsequent spreading in school. Although the percentage of TC was higher from January to June, this increase was not significant.

The main result from our retrospective analysis is that T. tonsurans displayed a continuous increase in the urban area of Paris in the general population and more specifically in patients of West African descent who, until recently, preferentially harboured the anthropophilic species M. audouinii and T. soudanense. Rarely reported in the early 2000’s, the importance of T. tonsurans has progressively increased in the Paris area. This correlates with a decrease in the rates of M. audouinii, while T. soudanense seems less impacted. These latter species remain the main causal agents of TC in West Africa. The predominance of T. tonsurans was also seen in Caribbean patients, many of whom are probably from Haiti (data not recorded) where T. tonsurans is known to be the major agent responsible for TC.

The current emergence of T. tonsurans as the main species causing TC is a reminder of what was reported in the 1980s, when the prevalence of T. tonsurans superseded that of M. audouinii in North America. Thus, M. audouinii progressively disappeared in the United States. Similarly, several authors reported T. tonsurans as the most prevalent causative agent of TC in London, United Kingdom, for the past 15 to 20 years, explained by the immigration in the United Kingdom from Caribbean islands. This immigration hypothesis may also apply to the phenomenon observed in the Paris area. Of note, although the Caribbean patients in the survey remained predominantly infected by T. tonsurans, T. soudanense was also recovered, suggesting transmission between communities, possibly through common hairdressings. Moreover, T. tonsurans is not reported in Africa, reinforcing the probability that the contamination took place in the Paris area.

The high propagation rate of T. tonsurans has been observed in France for many years, with reports of epidemics in paediatric care centers and in judo teams. A higher transmission of T. tonsurans cannot be explained by a higher number of carriers because we observed more carriers of M. audouinii than of T. tonsurans. Since the orally active drugs available mainly concentrate in the pilous bulb, treatments should be more active against endotrich species (mainly Trichophyton spp.) than against ectotrich species (mainly Microsporum spp.). With ectotrich species, external spores remain viable, hence the recommendation of antifungal shampoos to decrease the transmission of Microsporum spp. until mycological cure, and the recommendation of longer treatment for Microsporum.
spp. infection. In our cohort, we indeed observed more treatment failures for *M. audouinii* than for *Trichophyton* species. Taken together, the higher proportion of carriers, ectothrix hair parasitism, and delayed cure with antifungal treatment favours the expansion of *Microsporum* species instead of *Trichophyton* species. Thus, intrinsic features of *T. tonsurans* should explain its somewhat unexpected expansion. *Trichophyton* spp. have an arsenal of proteases capable of destroying keratin, such as keratinases, that were reported to have higher activity than those of *Microsporum* species. Whole genome sequencing and comparative analysis of seven dermatophyte species resulted in interesting differences between species. *Trichophyton tonsurans* has gained an additional copy of the subtilisin protease gene compared to the 12 subtilisins found in others sequenced dermatophytes. The M36 fungalytic genes of *T. tonsurans* also contains a predicted GPI anchor that is not found in other fungi, which could indicate an activity close to the cell wall. These differences could result in higher virulence in human hair and spreading success of *T. tonsurans*.

Another major point to explain the expansion of *T. tonsurans* is the exclusive use of griseofulvin, the only licensed paediatric drug in France for use against TC. One could wonder whether terbinafine, another drug of the armentarium against dermatophytes that is also orally available, could stop the dissemination of *T. tonsurans*. Several meta-analyses of randomized controlled trials did not show a significant difference in the overall efficacy of griseofulvin versus terbinafine, but efficacy differences were evidenced when considering the fungal species. For TC cases due to *Microsporum* spp., griseofulvin was superior (*P*= .04), whereas terbinafine was better as treating TC cases caused by *Trichophyton* spp. (*P*= .04). Another major issue to consider is the notorious bad compliance of griseofulvin treatment by children, especially when the treatment lasts 2 months.

In recent years, there have been few European studies investigating trends in TC epidemiology in European urban areas with this sample size. This 5-year study confirmed that *T. tonsurans* is expending in the Paris area, similar to what has already been reported in London and in the United States, pointing out the importance of continuing to record the species involved in TC. Although British and American guidelines support terbinafine as the first choice to treat *Trichophyton* TC, the French recommendation is griseofulvin since there is no paediatric formulation of terbinafine licensed in France. This could be challenged in the case of the continuous increase of *T. tonsurans*, at least to reduce the incidence of TC in children, as efficacy is better with terbinafine. In parallel, information about hygiene measures should be provided to decrease the rates of transmission through hairdressing. From a scientific point of view, basic studies are necessary to better understand the fitness of *T. tonsurans* as compared to other species.

**Supplementary material**

Supplementary data are available at *MMYCOL* online.

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**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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