

Epidemiological aspects and genotypic characterization of T.violaceum strains collected during a Belgian National survey on anthropophilic tinea



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Results

Objectives

To investigate the epidemiological determinants responsible for the high number of anthropophilic dermatophytes received by the National Reference Centre for Mycosis of Liege (NRCL), during the last years. Indeed, the last two years, clinical cases of tinea capitis caused by Microsporum audouinii (M. audouinii) and Trychophiton violaceum (T. violaceum), have increased in Belgium.

To perform a genotypic characterization by the **Diversilab®** system focusing on T.violaceum.

To present results of the national survey launched between February 2013 and March 2014.

Methods

Population: A total of 23 strains of T. violaceum (23 clinical+ 1 reference strains) collected between March 2013 and February 2014 were included in the study. The strains were collected from different laboratories through Belgium. Figure 1 describes the Diversilab® system (bioMérieux) used for genomic analysis of T. violaceum strains.



Step 2: Rep-PCR



Step 3: Diversilab® analysis





Figure 1: Description of the Diversilab® method.

Diversilab v3.4 PC Analosis Recent #173	Р	Sample ID	Genus	Species	Location	
1	1	13-130610-0104	Trichophyton	violaceum	Zute	
-2	1	13-130610-0105	Trichophyton	violaceum	Verrebroek	
1-3	1	13-130628-0102	Trichophyton	violaceum	BOCHOLT	
L4	1	13-130523-0057	Trichophyton	violaceum	DE PINTE	
r \$	1	13-1304 17-0083	Trichophyton	violaceum	HEVERLEE	
Le	1	13-130610-0106	Trichophyton	violaceum	ANVERS	
	1	13-130628-0100	Trichophyton	violaceum	HEVERLEE	
L.	1	13-130417-0084	Trichophyton	violaceum	Barsbeek	
[9	1	13-121022-0090	Trichophyton	violaceum	SAINT-GEORGES	
10	1	13-131220-0089	Trichophyton	violaceum	ANVERS	
- 11	1	13-130610-0102	Trichophyton	violaceum	Sint Andries	
□ ¹²	1	13-130426-0097	Trichophyton	violaceum	BRUXELLES	
- 13	1	13-130418-0196 1	Trichophyton	violaceum	BRUXELLES	
- 14	1	13-13041B-0176 1	Trichophyton	violaceum	BRUXELLES	
- 15	1	13-131220-0084	Trichophyton	violaceum	EVERGEN	
[_□ ¹⁶	1	3760 TV	Trichophyton	violaceum		
17	1	13-130314-0034	Trichophyton	violaceum	STEMBERT	
	1	13-120207-0185	Trichophyton	violaceum	STEMBERT	
_ ¹⁹⁺	2	13-130319-0285	Trichophyton	violaceum	DISON	
L20•	2	13-120210-0419	Trichophyton	violaceum	LIEGE	
21+	2	13-130419-00181	Trichophyton	violaceum	BRUXELLES	
2.	2	13-130418-0173 1	Trichophyton	violaceum	BRUXELLES	
23+	2	13-120621-0071	Trichophyton	violaceum	LIEGE	
24	2	13-131220-0090	Trichophyton	violaceum	BERCHEM	
s ex se se noo % Smilarity						

Figure 2: Dendrogram obtained after Diversilab® analysis of the strains N° 1 to 24

The 24 strains have been genotypically characterised by Diversilab® fingerprinting to visualize genomic variants between T.violaceum species. Figure 2 represents the DNA fingerprints of these strains (Strains 1 to 24).

Figure 3 represents the scatterplot of all strains analysed showing the differenciation into 2 different groups. The genotypic analysis led thus to the distinction of two genotypic variants of T.violaceum.



Figure 3: Scatter plots obtained after genomic analysis of strains by Diversilab® showing 2 different groups amongst T. violaceum strains.

The major group was composed of 17 strains which were mainly collected in the North of Belgium and included also the reference strain (18/23, 83,3%). The other group (6 strains) was close to the major group but the analysis of the spectral superposition showed some differences between these two groups, defining two distinct variants of *T. violaceum* in the Belgian population. This second variant was mainly recovered from South Belgium (5/6, 83,3%). Analysis of the epidemiological characteristics of the infected population shows that the main age category concerns 0-4 year-old children (n=9, 39,1%) with a sex-ratio M/F of 1.875. Data concerning the geographic origin of the family were present in 82,6% of the cases and reveal that patients were mainly of Ethiopian origin (n=8, 57,9% of known cases). One patient was also from Burundi showing that T. violaceum strains probably circulate mainly in East Africa.

	T. violaceum						
Variable	Origine géographique	N	Number (%)	p-value			
Province		23					
	Liège		6 (26.1)	0,31			
	Bruxelles		5 (21.7)				
	Brabant flamand		2 (8.7)				
	Limbourg		1 (4.3)				
	Anvers		4 (17.4)				
	Flandre occidentale		1 (4.3)				
	Flandre orientale		4 (17.4)				
	Hainaut		0 (0.0)				
	Brabant wallon		0 (0.0)				
Age (années)		23					
	0-4		9 (39.1)	0,13			
	5-9		8 (34.8)				
	10-14		4 (17.4)				
	>15		2 (8.7)				
Sexe		23					
	Femme		8 (34.8)	0,14			
	Homme		15 (65.2)				
Origine ethnique		12					
	Belgique		0 (0.0)	0,0039			
	Burundi		1 (8.3)				
	Congo		0 (0.0)				
	Guinée		0 (0.0)				
	Cameroun		0 (0.0)				
	Maroc		0 (0.0)				
	Mauritanie		0 (0.0)				
	Rwanda		0 (0.0)				
	Afrique du sud		0 (0.0)				
	силорів		11 (91.7)				

Table 1: Epidemiological analysis of the datas obtained during the national survey.

Conclusions

The DiversiLab® system proved to be an efficient method to investigate the molecular epidemiology of dermatophytes infections as reported previously for M. audouinii. These results show that two distinct isolates coexist in Belgium providing evidence of genetic heterogeneity and a possible spread of one genotypic variant in a restricted geographic area or the co-existence of two variants circulating in different African communities. However, no clear correlation established between could be the appartenance to a group and epidemiological factors, such as age or ethnical origin.