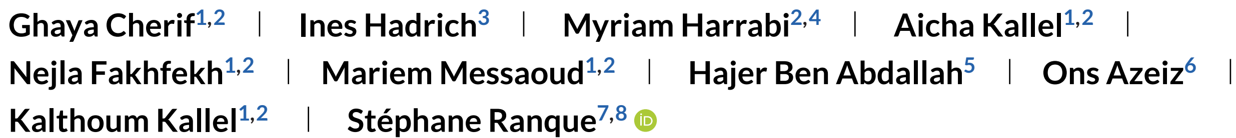
DOI: 10.1002/vms3.1015

ORIGINAL ARTICLE

WILEY

Aspergillus flavus genetic structure at a turkey farm



Ghaya Cherif[1,2] | Ines Hadrich[3] | Myriam Harrabi[2,4] | Aicha Kallel[1,2] | Nejla Fakhfekh[1,2] | Mariem Messaoud[1,2] | Hajer Ben Abdallah[5] | Ons Azeiz[6] | Kalthoum Kallel[1,2] | Stéphane Ranque[7,8]

[1]Laboratory of Parasitology and Mycology UR17SP 03, La Rabta Hospital, Tunis, Tunisia

[2]Higher Institute of Nursing Sciences of Tunis, University Tunis El Manar, Tunis, Tunisia

[3]Fungi and Parasitic Molecular Biology Laboratory, School of Medicine, University of Sfax, Sfax, Tunisia

[4]Laboratory of Bioinformatics, Biomathematics and Biostatistics UR16IPT09, Pasteur Institute of Tunis, Tunis, Tunisia

[5]Alfa Group Poultry Company, Chahia, Sfax, Tunisia

[6]Research Platform in Medicine Sciences and Technologies, Faculty of Medicine, Tunis, Tunisia

[7]Aix-Marseille Université, IRD, AP-HM, SSA, VITROME, Marseille, France

[8]IHU Méditerranée Infection, Marseille, France

Correspondence

Ines Hadrich, Fungi and Parasitic Molecular Biology Laboratory, School of Medicine, University of Sfax, Sfax, Tunisia.
Email: ineshadrich@yahoo.fr

Stéphane Ranque, Aix-Marseille Université, IRD, AP-HM, SSA, VITROME, Marseille, France.
Email: stephane.ranque@univ-amu.fr

Abstract

Background: The ubiquitous environmental fungus *Aspergillus flavus* is also a life-threatening avian pathogen.

Objectives: This study aimed to assess the genetic diversity and population structure of *A. flavus* isolated from turkey lung biopsy or environmental samples collected in a poultry farm.

Methods: *A. flavus* isolates were identified using both morphological and ITS sequence features. Multilocus microsatellite genotyping was performed by using a panel of six microsatellite markers. Population genetic indices were computed using FSTAT and STRUCTURE. A minimum-spanning tree (MST) and UPGMA dendrogram were drawn using BioNumerics and NTSYS-PC, respectively.

Results: The 63 environmental (air, surfaces, eggshells and food) *A. flavus* isolates clustered in 36 genotypes (genotypic diversity = 0.57), and the 19 turkey lung biopsies isolates clustered in 17 genotypes (genotypic diversity = 0.89). The genetic structure of environmental and avian *A. flavus* populations were clearly differentiated, according to both *F*-statistics and Bayesian model-based analysis' results. The Bayesian approach indicated gene flow between both *A. flavus* populations. The MST illustrated the genetic structure of this *A. flavus* population split in nine clusters, including six singletons.

Conclusions: Our results highlight the distinct genetic structure of environmental and avian *A. flavus* populations, indicative of a genome-based adaptation of isolates involved in avian aspergillosis.

KEYWORDS

aspergillosis, *Aspergillus flavus*, avian aspergillosis, Bayesian model, environment, *F*-statistics, genetic structure, *Meleagris gallopavo*, microsatellite markers, population genetics, typing

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2022 The Authors. *Veterinary Medicine and Science* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

Aspergillus spp. infections have been reported in almost all domesticated avian species (Seyedmousavi et al., 2015). Turkey poults have been reported to be particularly susceptible to aspergillosis with considerable economic damage due to mortality, decreased weight gain or condemnation at slaughter. Next to *Aspergillus fumigatus*, *Aspergillus flavus* is the predominant species involved in invasive aspergillosis (Arné et al., 2011). Despite the relatively scarce data available in the literature, *A. flavus* has been reported to be the main species implicated in both human aspergillosis and avian aspergillosis in Tunisia (Hadrich et al., 2013). *A. flavus* is an opportunistic fungal pathogen ubiquitously distributed, and abundant in favourable environments such as confinement buildings (Hadrich et al., 2013). Mildewed feed or contaminated litter are recognized sources of poultry exposure to *Aspergillus* spp. Moisture levels and dust-forming potential of the litter augment airborne fungal exposure and increase the risk of infection (Maciorowski et al., 2007). Thus, the relatively high temperature, humidity, dust and ammonia production levels present in poultry farms promote the rapid growth of hyphae and efficient asexual multiplication resulting in an abundant production of airborne hydrophobic conidia. These conidia are subsequently dispersed and inhaled by the birds incidence, and the risk of severe aspergillosis is correlated to the inoculum level (Arné et al., 2011).

Several genotypic studies have been used to study the population dynamics and transmissibility of Aspergillus species in several clinical and environmental settings, usually with a focus on hospital outbreaks (Sabino et al., 2014, 2019). Analysing the genetic structure of the strains involved in animal aspergillosis can also help one to trace their dissemination from the respiratory tract to other organs (Tsang et al., 2016). Understanding the distribution and relatedness of the pathogen is central to clarify aspergillosis epidemiology in avian farms and furthering the development of rational control strategies. Typing method may also give a deeper understanding of the colonization pattern of animals (Graham et al., 2008). However, limited studies, using molecular and statistical approaches, have been undertaken to assess the genetic pattern of *A. flavus* isolated from commercial turkey in Tunisia (Rodrigues et al., 2009). This study aimed at assessing genotypic polymorphisms to explore the population structure and the genetic relationship of avian and environmental *A. flavus* isolates to trace contamination sources of turkey aspergillosis cases in a commercial avian farm.

2 | MATERIALS AND METHODS

2.1 | Population study

Environmental (119) and clinical avian samples (38) from turkeys (*Meleagris gallopavo*) were collected at a poultry commercial farm in the governorate of Sfax, southeast of Tunisia, from February to May 2018, the season with the highest humidity in the environment.

Lung biopsies were aseptically taken from deceased chicks with lung lesions. Air samples were collected with the precipitation method (Williams et al., 2001), which consisted in a collection of airborne spores sedimented by gravity for 10–15 min onto the surface of a Sabouraud medium plate. Surface samples were taken by rubbing with a cotton swab. One gram of samples from the avian environment, including eggshells, food, and litter, were collected.

2.2 | Fungi isolation

One gram of broken eggshells, or litter or food sample or crushed biopsies, were put in a tube containing 10 ml of sterile saline solution. After shaking, 1 ml of suspension was inoculated onto a Sabouraud-chloramphenicol agar medium. The culture was incubated at 25°C for 4 days. The morphological identification of the colonies was based on both macroscopic and microscopic features.

2.3 | *A. flavus* molecular identification

The morphological identification of *A. flavus* isolates was confirmed by the rRNA ITS1–5.8S–ITS2 region sequence analysis, as reported by de Hoog et al. (2007). DNA was extracted by using a QIAmpkit (QIAGEN), following the manufacturer's instructions, and eluted with 50 μl of sterile water.

2.4 | Multilocus microsatellite typing (MLMT)

A. flavus isolates were genotyped by using six previously described (Hadrich et al., 2010) polymorphic microsatellite markers (AFLA1, AFLA3, AFLA7, AFPM3, AFPM4 and AFPM7) detailed in Table 1. FAM, NED and VIC fluorescent labels were used to distinguish the amplification products from distinct markers. PCR assays were performed in a final volume of 25 μl, containing 1 ng genomic DNA, 1 mM all amplification primers, 0.2 mM each dNTP, 3 mM $MgCl_2$ and 1 U AmpliTaq DNA Polymerase (Invitrogen) in 1× reaction buffer (Invitrogen). Thermocycling was performed in a T1 thermocycler (Biometra) using the following protocol: 5 min initial denaturation at 94°C, followed by 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 54°C and 30 s extension at 72°C, finally followed by 30 min at 72°C. PCR products were diluted 10-fold with formamide. One μl of diluted PCR products were combined with 15 μl formamide and a 0.5 μl LIZ-500 marker (Applied Biosystems). Following denaturation, the PCR products were resolved by capillary electrophoresis with polymer POP-4 in an ABI Prism 3130 genetic analyser (Applied Biosystems). Injection and running parameters followed the manufacturer's recommendations (Applied Biosystems). Analyses were performed with Gene Marker software (Applied Biosystems). The reproducibility of multilocus microsatellite typing (MLMT) was evaluated by using 5 different DNA preparations of the same isolate and by performing 10 repeated analyses of the same DNA preparation.

TABLE 1 Pattern of the six polymorphic microsatellite sequences of *Aspergillus flavus* up on analysis of 82 isolates

Primer name	Primer sequence (5′–3′)	Repeat unit	Fragment size	No. of alleles	*D*
AFLA1	CGTTGGCATGTTATCGTCAC CTACTGAATGGCGGGACCTA	AC	249–291	9	0.871
AFLA3	CTGAAAGGGTAAGGGGAAGG CACGCGAACTTATGGGACTT	TAGG	174–229	8	0.790
AFLA7	GCGGACACTGGATGAATAGC AACAAATCGGTGGTTGCTTC	TAG	261–354	5	0.557
AFPM3	CCTTTCGCACTCCGAGAC CACCACCAGTGATGAGGG	(AT)6AAGGGCG(GA)	199–217	6	0.692
AFPM4	AGCGATACAGTTTTAACACC TCTTGCTATACATATCTTCACC	CA	179–206	5	0.684
AFPM7	TGAGGCTGCTGTGGAACGC CAAATACCAATTACGTCCAACAAGGG	AC	215–276	10	0.903

2.5 | Genotypic data analysis

The population genetic parameter estimates were computed, and their statistical significance was tested with the FSTAT Version 2.9.3.2 software (Weir & Cockerham, 1984). The gene flow between populations was also assessed using FSTAT. In addition, data were analysed using a Bayesian model-based approach implemented in STRUCTURE, version 2.3.4 (Pritchard et al., 2000; Souto & Premoli, 2003). STRUCTURE uses Bayesian Monte Carlo Markov Chain sampling to identify the optimal number of clusters *K* for a given multi-locus dataset, without requiring a priori identification of the population subunits. The *K* optimal value (i.e., the optimal number of clusters in the dataset) was calculated using STRUCTURE HARVESTER, web version (Earl & vonHoldt, 2011). STRUCTURE was also used to identify migrants. The Simpson diversity index *D* was determined for each marker and possible markers combination (Hunter & Gaston, 1988). A minimum-spanning tree (MST) was built to illustrate the relationship between the genotypes with the BioNumerics (version 7.6) software (Applied Maths, Belgium). The degree of similarity was calculated by applying the Dice coefficient test, and an UPGMA dendrogram was generated, with the NTSYS-PC numerical taxonomy and multivariate analysis system (version2.1; Exeter Software, Setauket, NY, USA).

3 | RESULTS

3.1 | *A. flavus* was the most frequent fungal species

A. flavus was the most frequently isolated fungal species (65.62%) both in the environment (68.59% $n = 83$ out of 121 strains) and from avian biopsies (56.41% $n = 22$ out of 39 strains). It was followed by *Cladosporium* spp. (36.36%, $n = 20$ out of 55 strains), *Mucorales* (23.63%), *Aspergillus niger* (14.54%), *Alternaria* spp. (12.72%), *Penicillium* spp. (7.27%) and *A. fumigatus* (5.45%).

3.2 | Genetic diversity

Our findings showed an extremely high genetic diversity among avian isolates. The environmental isolates (ENV) included 36 genotypes (genotypic diversity = 0.57), and the clinical avian isolates (AVI) included 17 genotypes (genotypic diversity = 0.89). Upon the analysis of 82 isolates, 5–10 distinct alleles were detected for each microsatellite marker (Table 1). The six-marker combination generated 53 different haplotypes with a *D* value of 0.97.

3.3 | Genetic polymorphism

Genetic polymorphism was measured using allelic richness (*A*) and Nei's unbiased estimate of genetic diversity within sub-samples. Wright's *F* statistics were estimated using the Weir and Cockerham method. F_{IS} measures the relative inbreeding of individuals due to the local non-random union of gametes in each subpopulation. The comparison of the genetic diversity data for the two groups revealed that both allelic richness (*A*) intraspecific and genetic diversity (*Hs*) were higher in clinical avian ($A = 5.17$; $Hs = 0.711$) than in environmental ($A = 3.90$; $Hs = 0.396$) isolates. These results were confirmed by the inbreeding coefficient (F_{IS}) values estimated for each locus and in each population ($F_{IS} = 0.642$; $F_{IS} = 0.547$; $P \leq 0.05$).

3.4 | Inter-population genetic distance

The relative inbreeding in subpopulations, attributable to the subdivision of the total population into subpopulations of limited size, was estimated using the F_{ST} statistic. F_{ST} also measures the genetic differentiation between subpopulations. The avian environmental population was significantly differentiated from clinical avian population ($F_{ST} = 0.291[>0.25]$; $P = 0.05$).

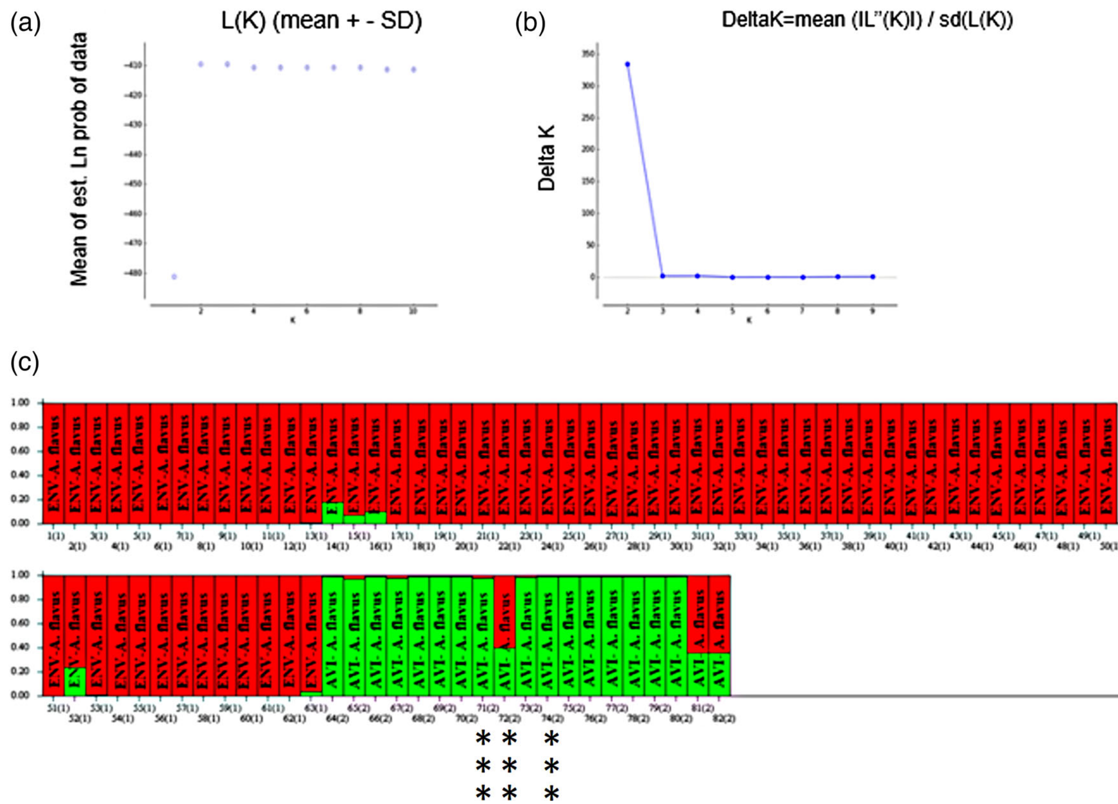


FIGURE 1 Estimated population structure of *Aspergillus flavus* in a commercial poultry farm in Sfax (Tunisia) as inferred by the STRUCTURE software on the basis of the data on six microsatellite markers obtained from 82 isolates from environment (ENV-*A. flavus*; $n = 63$) or turkey lung biopsy (AVI-*A. flavus*; $n = 19$) samples. (a) Plot of the mean posterior probability (Ln $P(D)$) values per clusters (K), based on 10 replicates per K, generated by the STRUCTURE software, and (b) ΔK analysis of Ln $P(K)$. (c) STRUCTURE plots grouped by Q-matrix (estimated membership coefficient for each sample) showing the distribution of genetic variation (c) at $K = 2$. Each strain is represented by a vertical line, which is partitioned into the coloured segments that represent the fungi estimated membership fractions in K. The same colour indicates that the isolates belong to the same group. Different colours for the same isolate indicate the percentage of the genotype shared with each group. An asterisk '*' marks isolates that appear misclassified by STRUCTURE, which identifies them as a possible migrant or a descendent of a recent immigrant from another population.

3.5 | Migration rate between environmental and clinical avian populations

The gene flow (Goudet, 1995), or migration rate, between avian environmental and clinical avian populations was relatively low, $Nm = 1 - F_{ST}/4F_{ST} = 0.609$.

3.6 | Bayesian model-based approach

The Bayesian model-based clustering analysis implemented in STRUCTURE indicated that our isolated set was most likely partitioned in two clusters (K) (maximum L [K] and maximum ΔK). Two approaches were used to choose K. First, the second-order rate of change in the log likelihood of the data between successive values of K (ΔK) was estimated. Second, posterior probabilities for the values of K with the highest Ln $P(X/K)$ were compared (Figure 1a,b). In agreement with the important genetic differentiation between populations, our analysis divided the population into two clusters according to their origin: ENV-*A. flavus* and AVI-*A. flavus* populations. For $K = 2$ (equal to the number of predefined populations), 98.9% of *A. flavus* isolates from the environment and 10.4% from avian clinical samples were included in one of the inferred clusters, and the remaining samples were included in the second cluster (Figure 1 and Table 2). Based on the Q-matrix bar plots obtained for each isolate by measuring the posterior probabilities of belonging to each K cluster, some strains belonging to ENV-*A. flavus* and AVI-*A. flavus* populations showed a mixed membership to the inferred clusters (Figure 1c). Then, the occurrence of gene flow between ENV-*A flavus* and AVI-*A. flavus* isolates was clearly illustrated in Figure 2. Furthermore, when running the migration model at $K = 2$, three isolates

TABLE 2 Proportion of membership of each pre-defined population in each of two clusters

Given pop	Inferred clusters		Number of individuals
	1	2	
1	0.989	0.011	63
2	0.104	0.896	19

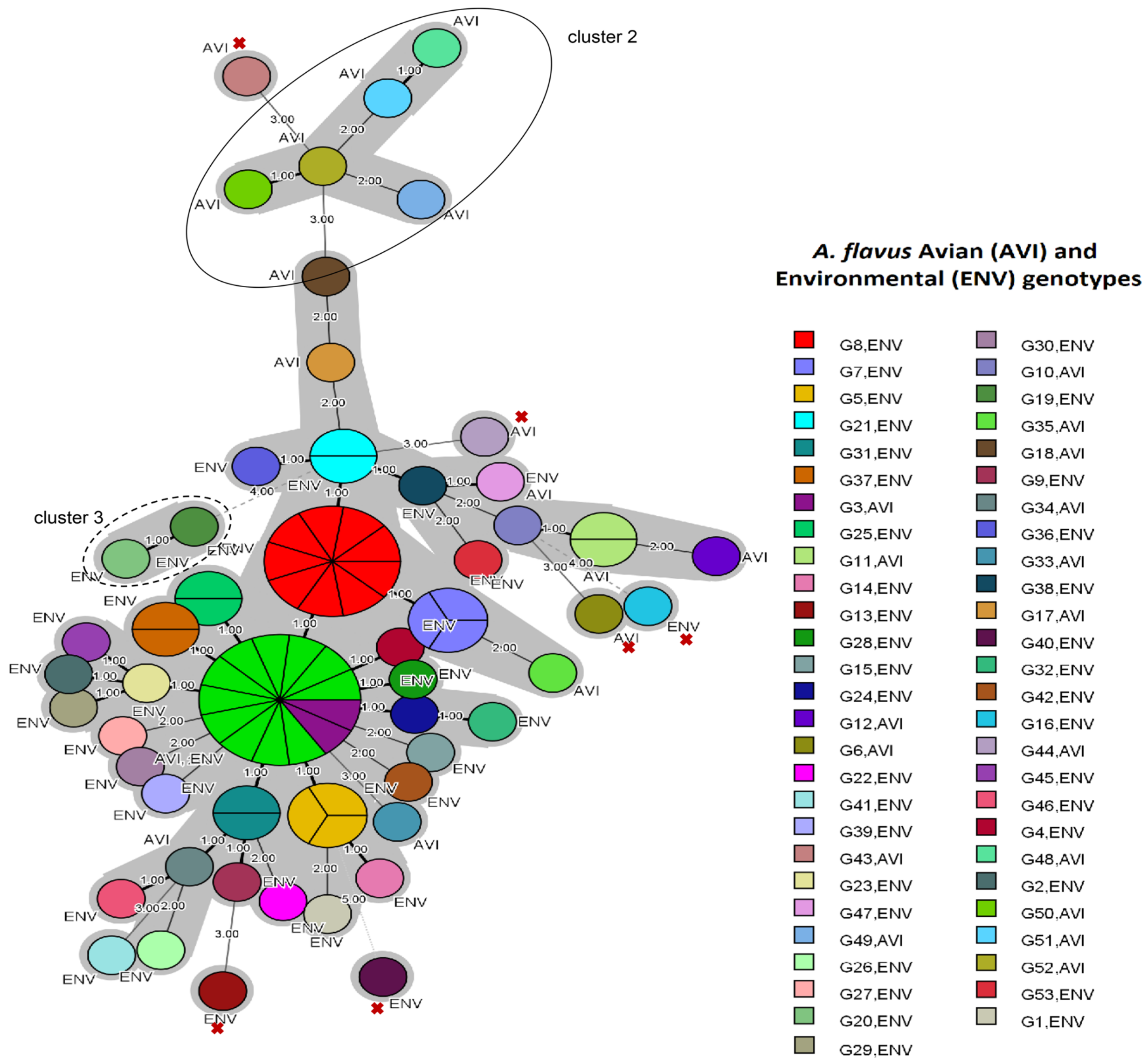


FIGURE 2 Minimum spanning tree showing the genotypic diversity of *Aspergillus flavus* isolates based on microsatellite data. *Each circle* shows a unique genotype and its size, the number of strains belonging to the same genotype. *Connecting lines and number* between *circles* show the similarity between genotypes: (*1.00*) indicates only one marker difference, (*2.00*) indicates difference in two in two markers, and (*3.00*) difference in three markers. '*' shows singletons.

from AVI-*A. flavus* population (lung biopsy) appeared misclassified and STRUCTURE identified them as a possible migrant and a descendent of a recent immigrant from ENV-*A. flavus* population over two generations according to Table 3. The three probabilities of being assumed to ENV-*A. flavus* population corresponded respectively to immigrant, parent immigrant and grandfather immigrant as mentioned in the column of 'probability of other pops' (Table 3).

3.7 | Genetic structure of *A. flavus* population

An MST analysis of the 53 distinct multilocus microsatellite genotypes was performed illustrate the genetic structure of our *A. flavus* population (Figure 2).

TABLE 3 Inferred ancestry of individuals: probability of being from assumed population probability of other populations

Sample ID	Probability of being from assumed population	Probability of other pops (immigrant parent and immigrant grandfather)
71	2: 0.002	Pop 1: 0.280 0.559 0.136***
72	2: 0.002	Pop 1: 0.310 0.619 0.069***
74	2: 0.002	Pop 1: 0.310 0.619 0.069***

****p* value <005

Genetic cluster analyses suggested that our *A. flavus* population was formed by nine genetic clusters; three different and distant clus-

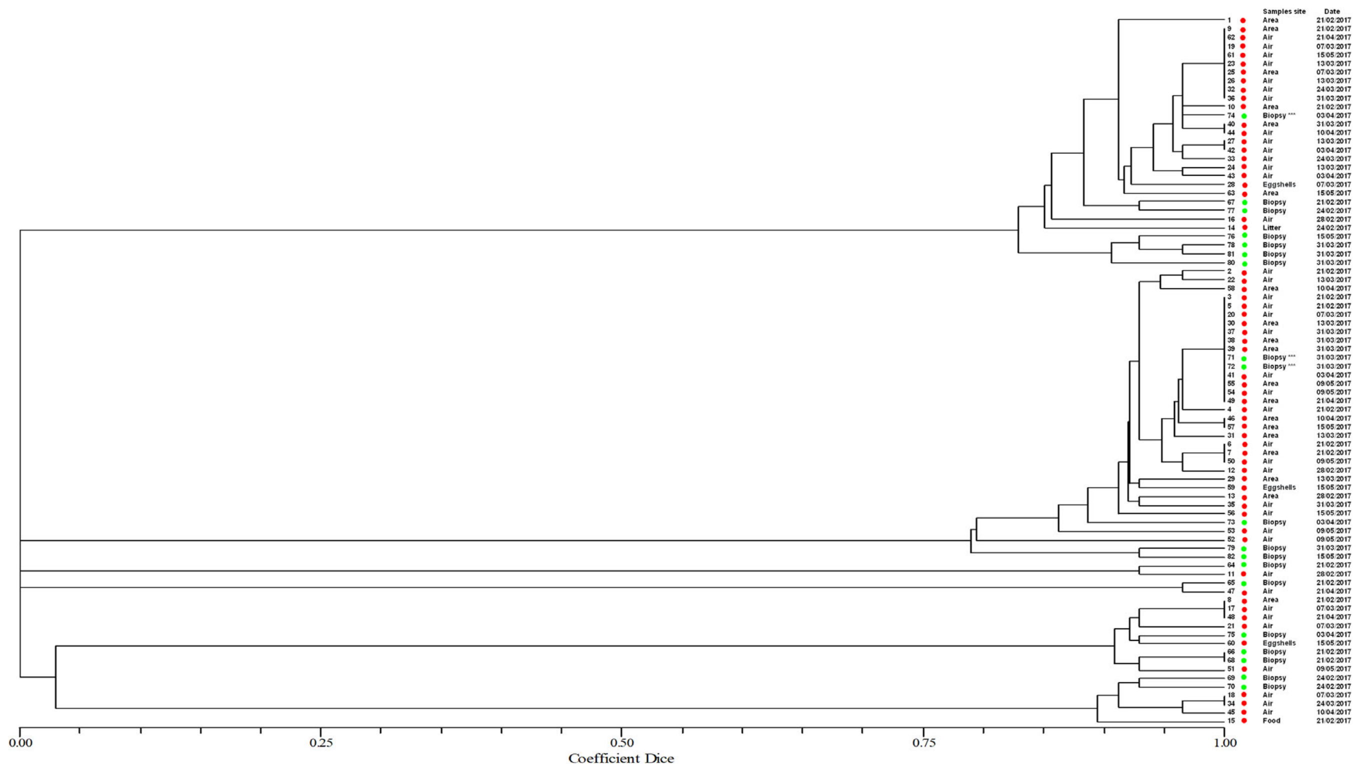


FIGURE 3 UPGMA dendrogram based on the Dice similarity coefficient upon analysis of six microsatellites markers obtained from 82 environmental (ENV-*Aspergillus flavus*; $n = 63$) and avian (AVI-*A. flavus*; $n = 19$) isolates. Two main clusters are identified. Each cluster encompasses both environmental (*red dots*) and avian isolates (*green dots*) without any strict partition according to sampling origin. The three migrants isolates (lung biopsy) appear with triple stars '*'.

ters indicate differences in three or more markers. The first cluster was dominated by strains from the environmental isolates (34 ENV-*A. flavus* genotypes vs. 6 AVI-*A. flavus* genotypes). The second cluster comprised five AVI-*A. flavus* isolates. The third cluster comprised two ENV-*A. flavus* isolates (Figure 2).

Cluster 1 (34 ENV-*A. flavus* genotypes vs. 6 AVI-*A. flavus* genotypes) and cluster 2 (5 AVI-*A. flavus* isolates) differed by 3 markers. Cluster 1 (34 ENV-*A. flavus* genotypes vs. 6 AVI-*A. flavus* genotypes) and cluster 3 (2 ENV-*A. flavus* isolates) differed by 4 markers (Figure 2).

Six unique genotypes formed singletons; five genotypes (three ENV-*A. flavus* genotypes vs. two AVI-*A. flavus* genotypes) clustered together and with the cluster 1. One AVI-*A. flavus* genotype clustered with the cluster 2 (Figure 2).

3.8 | Phylogenetic analyses

The phylogenetic tree construction included two main clusters, and the first was divided into two sub-clusters. Pairwise Dice coefficient of similarity values between strains ranged from 0 to 1 (Figure 3). The Dice value was 1 for cases with multiple ENV-*A. flavus* strains. An identical genotype was found in 2 clinical avian isolates (71-72) and 11 environmental isolates (3-5-20-30-37-38-39-41-55-54-49) suggesting that these latest ones could also be responsible of avian aspergillosis. Four AVI-*A. flavus* isolates were related to each other (76-78-81-80). Three AVI-*A. flavus* isolates (75-66-68) were highly similar to isolates collected from Egg samples (60) and eight AVI-*A. flavus* isolates (73-79-82-64-65-69-70-75) were related to isolates collected from air samples (56-53-52-11-47-17-48-21).

4 | DISCUSSION

A. flavus causes aspergillosis worldwide, with a higher incidence in tropical countries. It causes aspergillosis outbreaks in poultry farms; and turkeys are at particularly high risk. By using MLMT, we highlighted the high genetic diversity of *A. flavus* population, the normalized indices of association indicated a predominant clonal reproduction mode, and a migration flow between *A. flavus* isolates collected in the same commercial poultry farm. The *F*-statistics indicated historical migration events and the Bayesian approach weighted towards probable recent migration events, which advocates for the hypothesis that turkeys' aspergillosis resulted of the spreading of a population, rather than of a small set of genotypes, of environmental *A. flavus* isolates (Chen et al., 2015). Both the Bayesian model-based clustering analysis implemented in STRUCTURE and the multilocus microsatellites genotypes patterns clearly separated the environmental from the clinical avian isolates. This separation pattern could, at least in part, be due the relatively low number of time-point measured within each population, the relatively short time period studied and the relatively homogenous

geographical area studied. Indeed, the VNTR clustering analysis of 55 Chinese avian farms *A. flavus* isolates showed a significant differentiation regarding their geographic origin (Wang et al., 2012). Further studies are needed to assess and compare the genetic variability of environmental and avian population of *A. flavus* in commercial avian farms. Our findings suggest that the contamination source is the environment. Because of their high mutation rate, and allele homoplasy resulting from reverse mutations, the use of microsatellites as genetic marker is prone to underestimate subpopulation divergence (F_{ST} values), the gene flow, and migration rate between the environmental and the clinical isolates populations (Rouger et al., 2017). Single-nucleotide polymorphism loci are less susceptible to these effects, they may provide better estimates of F_{ST} and may give more relevant information on reproduction (Lorenz et al., 2010).

In agree with a previous study (Hadrich et al., 2013) conducted on a smaller sample in Tunisia, we assessed that *A. flavus* was the chief avian aspergillosis agent in the Sfax region. We also confirmed the gene flow and the presence of clear separation between avian and environmental populations. This study provided data on the diversity of the *A. flavus* contaminating avian farms, their population structure and the genetic relationship between strains. Our findings supported that strengthening the management of laying hen farms, which includes the control of the temperature and humidity, and appropriate cleaning procedures, is mandatory to control the dispersion of airborne *Aspergillus* conidia, to reduce the risk of animal infection, and to improve the hygienic quality and safety of table eggs (Cafarchia et al., 2014; Rath, 2001; Singh, 2015; Thierry et al., 2010).

AUTHOR CONTRIBUTIONS

Ghaya Cherif: formal analysis (equal), investigation (lead), writing – original draft (lead); Ines Hadrich: conceptualization (lead), investigation (equal), formal analysis (lead), supervision (lead), validation (equal), writing – review and editing (equal); Myriam Harrabi: methodology (equal), validation (equal), writing – original draft (equal); Aicha Kallel: investigation (equal), methodology (equal), writing – review and editing (equal); Nejla Fakhfekh: investigation (equal), writing – original draft (equal); Mariem Messaoud: investigation (equal), resources (equal), writing – original draft (equal); Hajer Ben Abdallah: investigation (equal), resources (equal); Ons Azeiz: methodology (equal), resources (equal); Kalthoum Kallel: investigation (equal), supervision (equal), writing – review and editing (equal); Stéphane Ranque: validation (lead), writing – review and editing (lead).

ACKNOWLEDGEMENTS

We are grateful to Dr. Maher Kharrat for the genotyping work using the ABI 3500 Genetic Analyzer (Plateforme de Recherche, Faculté de Médecine de Tunis).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

FUNDING INFORMATION

None to mention.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is an original research conducted on microscopic fungi, neither on human nor on animals.

ORCID

Stéphane Ranque https://orcid.org/0000-0003-3293-5276

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.1015.

REFERENCES

Arné, P., Thierry, S., Wang, D., Deville, M., Le Loc'h, G., Desoutter, A., Féménia, F., Nieguitsila, A., Huang, W., Chermette, R., & Guillot, J. (2011). *Aspergillus fumigatus* in poultry. *International Journal of Microbiology*, *2011*, 746356.

Cafarchia, C., Camarda, A., Iatta, R., Danesi, P., Favuzzi, V., Di Paola, G., Pugliese, N., Caroli, A., Montagna, M. T., & Otranto, D. (2014). Environmental contamination by *Aspergillus spp*. in laying hen farms and associated health risks for farm workers. *Journal of Medical Microbiology*, *63*(Pt 3), 464–470.

Chen, Y., Litvintseva, A. P., Frazzitta, A. E., Haverkamp, M. R., Wang, L., Fang, C., Muthoga, C., Mitchell, T. G., & Perfect, J R. (2015). Comparative analyses of clinical and environmental populations of *Cryptococcus neoformans* in Botswana. *Molecular Ecology*, *24*(14), 3559–3571.

de Hoog, G. S., Nishikaku, A. S., Fernandez-Zeppenfeldt, G., Padín-González, C., Burger, E., Badali, H., Richard-Yegres, N., & van den Ende, A. H. (2007). Molecular analysis and pathogenicity of the *Cladophialophora carrionii* complex, with the description of a novel species. *Studies in Mycology*, *58*, 219–234.

Earl, D. A., & vonHoldt, B. M. (2011). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361.

Goudet, J. (1995). FSTAT (Version 1.2): A computer program to calculate F-statistics. *Journal of Heredity*, *86*(6), 485–486.

Graham, J. P., Leibler, J. H., Price, L. B., Otte, J. M., Pfeiffer, D. U., Tiensin, T., & Silbergeld, E. K. (2008). The animal-human interface and infectious disease in industrial food animal production: Rethinking biosecurity and biocontainment. *Public Health Reports*, *123*(3), 282–299.

Hadrich, I., Amouri, I., Neji, S., Mahfoud, N., Ranque, S., Makni, F., & Ayadi, A. (2013). Genetic structure of *Aspergillus flavus* populations in human and avian isolates. *European Journal of Clinical Microbiology & Infectious Diseases*, *32*(2), 277–282.

Hadrich, I., Drira, I., Neji, S., Mahfoud, N., Ranque, S., Makni, F., & Ayadi, A. (2013). Microsatellite typing of *Aspergillus flavus* from clinical and environmental avian isolates. *Journal of Medical Microbiology*, *62*(Pt 1), 121–125.

Hadrich, I., Makni, F., Ayadi, A., & Ranque, S. (2010). Microsatellite typing to trace *Aspergillus flavus* infections in a hematology unit. *Journal of Clinical Microbiology*, *48*(7), 2396–2401.

Hunter, P. R., & Gaston, M. A. (1988). Numerical index of the discriminatory ability of typing systems: An application of Simpson's index of diversity. *Journal of Clinical Microbiology*, *26*(11), 2465–2466.

Lorenz, A. J., Hamblin, M. T., & Jannink, J. L. (2010). Performance of single nucleotide polymorphisms versus haplotypes for genome-wide association analysis in barley. *PLoS One*, *5*(11), e14079.

Maciorowski, K. G., Herrera, P., Jones, F. T., Pillai, S. D., & Ricke, S. C. (2007). Effects on poultry and livestock of feed contamination with bacteria and fungi. *Animal Feed Science and Technology*, *133*(1–2), 109–136.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data Genetics. *155*(2), 945–959.

Rath, P. M. (2001). Phenotypic and genotypic characterization of reference strains of the genus *Aspergillus*. *Mycoses*, *44*(3–4), 65–72.

Rodrigues, P., Venancio, A., Kozakiewicz, Z., & Lima, N. (2009). A polyphasic approach to the identification of aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* Section *Flavi* isolated from Portuguese almonds. *International Journal of Food Microbiology*, *129*(2), 187–193.

Rouger, A., Tresse, O., & Zagorec, M. (2017). Bacterial contaminants of poultry meat: Sources, species, and dynamics. *Microorganisms*, *5*(3), 50.

Sabino, R., Burco, J., Valente, J., Veríssimo, C., Clemons, K. V., Stevens, D. A., & Tell, L. A. (2019). Molecular identification of clinical and environmental avian *Aspergillus* isolates. *Archives of Microbiology*, *201*(2), 253–257.

Sabino, R., Veríssimo, C., Parada, H., Brandão, J., Viegas, C., Carolino, E., Clemons, K. V., & Stevens, D. A. (2014). Molecular screening of 246 Portuguese *Aspergillus* isolates among different clinical and environmental sources. *Medical Mycology*, *52*(5), 519–529.

Seyedmousavi, S., Guillot, J., Arné, P., de Hoog, G. S., Mouton, J. W., Melchers, W. J., & Verweij, P. E. (2015). *Aspergillus* and aspergilloses in wild and domestic animals: A global health concern with parallels to human disease. *Medical Mycology*, *53*(8), 765–797.

Singh, N. D. (2015). Aspergillosis in avian species: A review. *Journal of Poultry Science and Technology*, *3*(1), 1–14.

Souto, C. P., & Premoli, A. C. (2003). Genetic divergence among natural populations of *Alstroemeria aurea* D. Don: A dominant clonal herb of the understory in subalpine *Nothofagus* forests. *Botanical Bulletin of Academia Sinica*, *44*, 329–336.

Thierry, S., Wang, D., Arné, P., Deville, M., De Bruin, B., Nieguitsila, A., Pourcel, C., Laroucau, K., Chermette, R., Huang, W., Botterel, F., & Guillot, J. (2010). Multiple-locus variable-number tandem repeat analysis for molecular typing of *Aspergillus fumigatus*. *BMC Microbiology*, *10*, 315.

Tsang, C. C., Hui, T. W., Lee, K. C., Chen, J. H., Ngan, A. H., Tam, E. W., Chan, J. F., Wu, A. L., Cheung, M., Tse, B. P., Wu, A. K., Lai, C. K., Tsang, D. N., Que, T. L., Lam, C. W., Yuen, K. Y., Lau, S. K., & Woo, P. C. (2016). Genetic diversity of *Aspergillus* species isolated from onychomycosis and *Aspergillus hongkongensis* sp. nov., with implications to antifungal susceptibility testing. *Diagnostic Microbiology and Infectious Disease*, *84*(2), 125–134.

Wang, D., Gricourt, M., Arné, P., Séguin, D., Chermette, R., & Huang, W., et al. (2012). May avian farms constitute a source of azole resistant *Aspergillus fumigatus*? 5th advances against aspergillosis Istanbul Turkey. 8.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution; International Journal of Organic Evolution*, *38*(6), 1358–1370.

Williams, R. H., Ward, E., & McCartney, H. A. (2001). Methods for integrated air sampling and DNA analysis for detection of airborne fungal spores. *Applied and Environmental Microbiology*, *67*(6), 2453–2459.

How to cite this article: Cherif, G., Hadrich, I., Harrabi, M., Kallel, A., Fakhfekh, N., Messaoud, M., Abdallah, H. B., Azeiz, O., Kallel, K., & Ranque, S. (2023). *Aspergillus flavus* genetic structure at a turkey farm. *Veterinary Medicine and Science*, *9*, 234–241. https://doi.org/10.1002/vms3.1015