

Non-Tuberculous Mycobacteria As Environmental Risk Factors For Human Infectious Diseases – Isolation Of Non-Tuberculous Mycobacteria From Patients In The Thessaly Region (Central Greece) And Their Correlation With Sarcoidosis Disease

E Dovriki, F Bardaka, I Gerogianni, E Petinaki, K I Gourgoulisanis, A Papaioannou

Citation

E Dovriki, F Bardaka, I Gerogianni, E Petinaki, K I Gourgoulisanis, A Papaioannou. *Non-Tuberculous Mycobacteria As Environmental Risk Factors For Human Infectious Diseases – Isolation Of Non-Tuberculous Mycobacteria From Patients In The Thessaly Region (Central Greece) And Their Correlation With Sarcoidosis Disease*. The Internet Journal of Microbiology. 2018 Volume 15 Number 1.

DOI: [10.5580/IJMB.52861](https://doi.org/10.5580/IJMB.52861)

Abstract

Mycobacteria are organisms that are of great interest in the field of medicine, particularly those causing tuberculosis and leprosy (e.g., mycobacterium tuberculosis). Conversely, the nontuberculous mycobacteria (NTM) that have been found to be widely dispersed in the environment are considered potentially pathogenic for humans and animals. Recently, NTM found in patients were correlated with sarcoidosis disease. The aim of the present study is to identify atypical mycobacteria, isolated from various samples of patients, and examine their possible correlation with sarcoidosis disease, for the period of 2010 to 2016. A total of 296 positive NTM patients (60% male) have been included in the study. Seventeen different NTM species were identified from the studied biological samples. The main species distribution among patients were as follows; *M. fortuitum* (n=107); *M. gordonae* (n=61); *M. chelonae* (n=44); *M. intracellulare* (n=27); *M. avium* (n=16) and *M. abscessus* (n=13). At the same period of time, a total of 124 sarcoidosis cases have been recorded from the same geographical area. The results of the present study showed that indeed there is a large dispersion of positive NTM patients in the environment. In addition, regarding the geographical distribution of positive NTM patients and patients with sarcoidosis disease in the same study area, there is evidence of a possible correlation between them.

1. INTRODUCTION

It is known worldwide that mycobacteria, which cause tuberculosis and leprosy (e.g. *M. Tuberculosis*), are not detected in the environment and are transmitted from human to human. Today, mycobacteria are of great interest to medicine industry because of human migration.

On the other hand, nontuberculous mycobacteria (NTM) that have been found to be widely dispersed in the environment are considered potentially pathogenic for humans and animals. Although NTM cause diseases in animals (e.g. *M. avium* - *intracellulare* causes disease in poultry and pigs), animal to human transmission is rare. Until today, more than 160 different species of NTM have been isolated and

identified, while this number is increasing continuously due to improved determination and identification techniques of biological and environmental samples [Dantec et al. 2002; Epton and Einthrop 2012]. In the scientific literature, they are referred to as potential pathogens principally to immunosuppressed and immunocompetent patients (e.g. transplant recipients, AIDS and cancer patients), as well as to vulnerable groups such as children, chronically ill, and the elderly. Generally, populations at risk include individuals who suffer from lung disease and a weakened immune system. [Archibald and Jarvis 2011; Billinger et al. 2009; Epton and Einthrop 2012; Gerogianni et al. 2008; Gitti et al. 2011; Moore et al. 2010; Moorthy et al. 2012; Penn et al. 2011; Roux et al. 2009; Russell et al. 2014; Chou et al.

2014]. Only few sporadic studies have been published, mainly showing that NTM cause lymphadenitis and infections to humans, mostly in the developed world, with increased frequency not geographically delimited. This increased incidence may be attributed to the interest obtained by the researchers, mainly due to AIDS patients with NTM infections. It is estimated that 25-50% of patients with AIDS have been infected by NTM [Dantec et al. 2002; Falkinham III 2009 and 2011; Gerogianni et al. 2008; Gill et al. 2011; Gitti et al. 2011; Moore et al. 2010; Griffith et al. 2007; Iseman et al. 2008; Jolobe 2011; Kaevska and Hruska 2010; Kankya et al. 2011; Lyu et al. 2011; Maekawa et al. 2011].

Another area of interest proved to be the study of possible correlation between the presence of NTM in patients and sarcoidosis disease, whereas recent research data suggest that there is a correlation between them [Ainslie and Benatar 1985; Olive and Kataria 1985; James 1986; James 1992; Oksanen 1994; Kon and Du Bois 1997; Sharma 1997; Am J Respir Crit Care Med. 1999; Dumouchel-Champagne et al. 2009; Keijsers et al. 2009; Costabel et al. 2010; Iannuzzi and Fontana 2011; Tavee and Culver 2011; Drent et al. 2012; Sobic-Saranovic et al. 2013; Blankstein et al. 2014; Cremers et al. 2014; Gupta et al. 2014; Mortaz et al. 2014]

Multivariate statistical methods are useful tools to examine possible correlations between different parameters and factors, as they give results when classical correlation analysis methods cannot. The use of these methods to investigate the association between NTM isolated in humans with other parameters has been considered crucial and necessary. Specifically, the use of Cluster Analysis and Factor Analysis to analyze large data sets in medicine research has proven valuable, giving results that the classical statistical analysis methods are not able to trace [Massart and Kaufman 1983; Vanderginste et al. 1998; Poupard et al. 2002; Vogt and Nagel 1992; Nguyen et al. 2003; Ness et al. 2005; Papaioannou et al. 2010].

In the present study, multivariate statistical methods have been used to examine possible correlations among the parameters “NTM” isolated from human patients, “SEX” of positive NTM patients, “CLINIC” that came the positive NTM biological sample, “BIOSAMPLE” the kind of positive NTM biological sample and “RESIDENCE” of positive NTM patients”. Furthermore, for the first time in Greece, the present study investigates the possible

correlation between the NTM isolated from patients and the cases of sarcoidosis disease, in the Thessaly region, Greece.

2. METHODS

2.1 General

Methods that were used for digestion and decontamination of clinical samples to recover from *M. tuberculosis* have also proved to be useful for the NTM. After sample preparation (liquidation, homogenization, decontamination and neutralization), staining and microscopy take place. Once decontaminated, samples may be grown on ordinary solid media for mycobacteria such as egg-based Lowenstein-Jensen (LJ), agar based medium (Middlebrook) or selective media (LJ Gruft; Difco Laboratories, Oxford, UK). Liquid media cultures include the automated Bactec MGIT 960 [Dantec et al. 2002; Gitti et al. 2011].

Sarcoidosis diagnosis was based on a compatible clinical and radiological images, the presence of non-tyroidized granulomas in tissue biopsies and the exclusion of other granulomatous diseases [Ainslie and Benatar 1985; Olive and Kataria 1985; James 1986; James 1992; Oksanen 1994; Kon and Du Bois 1997; Sharma 1997; Am J Respir Crit Care Med. 1999; Dumouchel-Champagne et al. 2009; Keijsers et al. 2009; Costabel et al. 2010; Iannuzzi and Fontana 2011; Tavee and Culver 2011; Drent et al. 2012; Sobic-Saranovic et al. 2013; Blankstein et al. 2014; Cremers et al. 2014; Gupta et al. 2014; Mortaz et al. 2014]

2.2 NTM cases

The study included patients admitted to the University Hospital of Larissa, from whom at least one biological sample was tested culture-positive for NTM. The study was approved by the Ethics Committee of the University of Thessaly. All patients were informed and provided their written consent.

NTM isolates were recovered from respiratory specimens, urine, sterile body fluids, and tissues. Multiple identical isolates related to one single individual, who was living at the same site and who was hospitalized during the same period, were counted as a one patient entry.

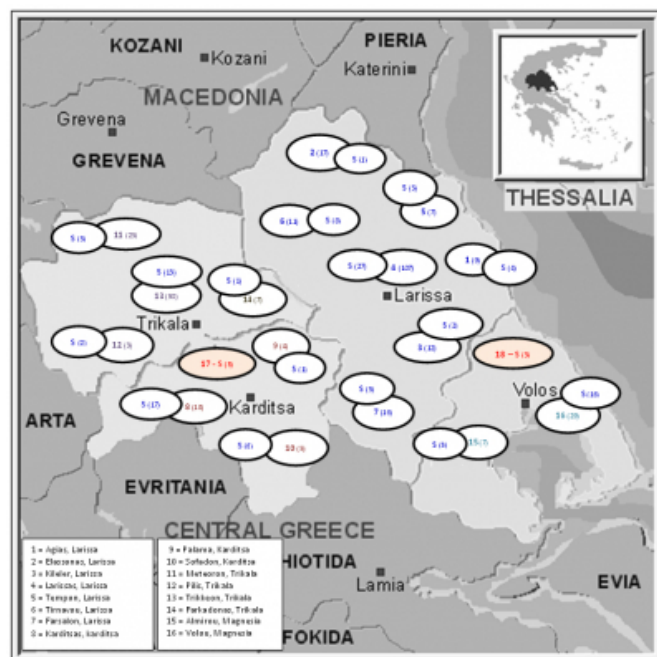
A total of 302 NTM species were isolated in the department of Microbiology, University Hospital of Larissa. All isolates included in the study were recovered from 296 patients who lived in the Thessaly region, central Greece, during a 7-year

Non-Tuberculous Mycobacteria As Environmental Risk Factors For Human Infectious Diseases – Isolation Of Non-Tuberculous Mycobacteria From Patients In The Thessaly Region (Central Greece) And Their Correlation With Sarcoidosis Disease

period from January 2010 to December 2016. Subsequently, the medical records of these patients were reviewed, aiming to identify their locality of residence and relevant clinical characteristics. It was found that these individuals lived in 16 different locations in the region of Thessaly. The majority of them lived in the cities of Larissa, Trikala, Karditsa and Volos. The geographical distribution of these locations is presented in Figure 1.

Figure 1

Map of Thessaly region with the studied localities (municipalities) (Prefecture of Larissa: 1 = Agia; 2 = Elassona; 3 = Kileler; 4 = Larissa; 5 = Tempi; 6 = Tirnavos; 7 = Farsala. Prefecture of Karditsa: 8 = Karditsa; 9 = Palama; 10 = Sofades; 17 = Mouzaki. Prefecture of Trikala: 11 = Meteora; 12 = Pyli; 13 = Trikkaion; 14 = Farkadona. Prefecture of Magnesia: 15 = Almyros; 16 = Volos; and 18 = Riga Feraios).



2.3 Clinical specimens processing

Clinical specimens were stained by Ziehl-Nielsen and were grown as well. In more detail, a volume of 0.5 ml was inoculated into a Bactec MGIT 960 tube, while 0.25 ml were inoculated onto a commercially available Lowenstein-Jensen medium (BioMerieux, France). The Lowenstein-Jensen medium was incubated at 37°C and routinely examined for up to 6 and 8 weeks respectively.

All positive cultures were subsequently analyzed by the Genotype CM commercial kit (HainLife Science, Germany),

a molecular genetic assay for identification of M. tuberculosis complex and 15 of the most common NTM species (Mycobacterium avium, Mycobacterium chelonae, Mycobacterium abscessus, Mycobacterium fortuitum, Mycobacterium gordonae, Mycobacterium intracellulare, Mycobacterium scrofulaceum, Mycobacterium interjectum, Mycobacterium kansasii, Mycobacterium malmoeense, Mycobacterium peregrinum, Mycobacterium marinum / Mycobacterium ulcerans, Mycobacterium xenopi and MTBC). This assay is a recently developed commercial DNA-strip one about differentiating mycobacteria isolates from cultured media.

2.4 Sarcoidosis Cases

The study included 124 patients admitted to the University Hospital of Larissa and diagnosed with sarcoidosis disease for the same time period. The study was approved by the Ethics Committee of the University of Thessaly. All patients were informed and provided their written consent. Figure 1 presents the place of residence of positive NTM patients as well as the number of the sarcoidosis cases in Thessaly region for the period 2010 - 2016.

2.5 Statistical Analysis by Chemometric Methods

Two major classes of chemometric methods can be distinguished. The first comprises the methods concerned with the recognition of patterns (e.g. linear discriminant analysis). The second class of methods aims at the detection of patterns yet unnoticed in the field of medicine. A single pattern may be noticed, recognized as a highly abnormal one. Or a group of patterns belonging to a major category of patterns may be delimited, because the patterns of this group are in some sense distinct from all other patterns of the category – they form a “cluster”.

Cluster Analysis (CA) and Factor Analysis (FA) were used for multivariate statistical modeling of the input data. The calculation work was performed by the use of software package STATISTICA 10.0 for Windows.

1. Cluster Analysis (CA)

CA is a data reduction method that is used to classify entities with similar properties. The method divides a large number of objects into a smaller number of homogeneous groups on the basis of their correlation structure. The objective of CA is to identify the complex nature of multivariate relationships (by searching for natural groupings or types)

among the data under investigation, so as to foster further hypothesis development about the phenomena being studied. CA imposes a characteristic structure on the data analysis for exploratory purposes [Massart and Kaufman 1983; Vanderginste et al. 1998; Poupard et al. 2002; Vogt and Nagel 1992; Ness et al. 2005; Papaioannou et al. 2009].

B. Factor Analysis (FA)

FA is used to find associations between parameters, so that the number of measured parameters can be reduced. Known associations are then used to predict unmeasured parameters [Vanderginste et al. 1998; Poupard et al. 2002; Nguyen et al. 2003; Papaioannou et al. 2009].

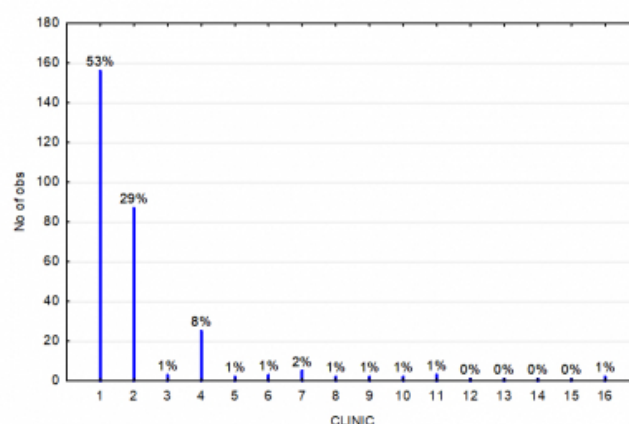
FA is often used in data reduction to identify a small number of factors that explain most of the variance observed in a much larger number of manifest variables. Principal Component Analysis (PCA), as one subset of FA, extracts eigenvalues and eigenvectors (a list of loadings) from the covariance matrix of original variables to produce new orthogonal variables known as VariFactors (VFs), usually through Varimax rotation, which are linear combinations of the original variables. VFs provide information about the most meaningful parameters that describe a whole data set allowing data reduction with minimum loss of original information. Moreover, VFs include unobservable, hypothetical, latent variables.

3. RESULTS

The source of the NTM isolates were respiratory specimens from the hospital's pulmonary clinic (53%), samples from the pathological clinic (30%), the rheumatology clinic (8%), and the gastrointestinal clinic (1.0%), while the remaining of the samples came from other clinics, presented in Figure 2.

Figure 2

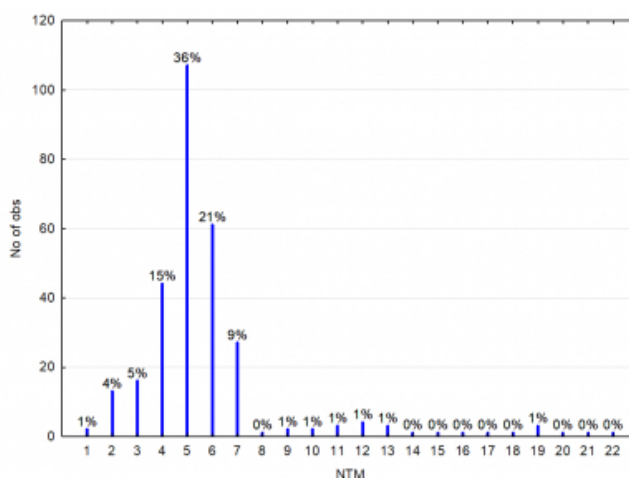
Frequency of NTM positive patients per clinic



In the NTM isolates from the clinical specimens (Figure 3), the most frequently isolated organisms were *M. fortuitum* (n=107, 36%), *M. gordonae* (n=61, 21%), *M. chelonae* (n=44, 15%); *M. intracellulare* (n=27, 9%), *M. avium* (n=16, 5%) and *M. abscessus* (n=13, 4%). Additionally, *M. tuberculosis* was isolated in 1 patient. It is noteworthy that in 6 patients isolated 2 NTM species simultaneously.

Figure 3

Frequency of NTM species found in clinical specimens (1 = *M. lentiflavum*; 2 = *M. abscessus*; 3 = *M. avium*; 4 = *M. chelonae*; 5 = *M. fortuitum*; 6 = *M. gordonae*; 7 = *M. intracellulare*; 8 = *M. kansasii*; 9 = *M. arupense*; 10 = *M. conceptionense*; 11 = *M. peregrinum*; 12 = *M. smegmatis*; 13 = *M. chimaera*; 14 = *M. mucogenicum*; 15 = *M. yongonense*; 16 = *M. simiae*; 17 = *Mycobacterium* spp; 18 = *M. canariense*; 19 = *M. avium* & *M. intracellulare*; 20 = *M. chelonae* & *M. gordonae*; 21 = *M. avium* & *M. chelonae*; 22 = *M. fortuitum* & *M. chelonae*).

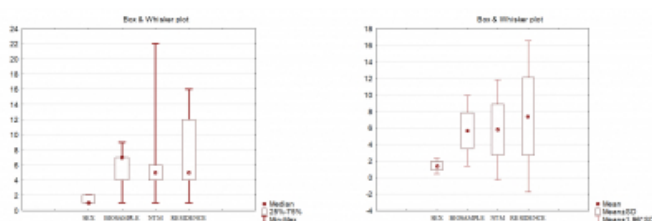


3.1 Box and Whisker plot analysis

When Box & Whisker plot analysis is conducted for all data set (Figure 4), it can be seen that the parameters “BIOSAMPLE”, “NTM” and “RESIDENCE” correlated each other.

Figure 4

Frequency of NTM species found in clinical specimens (1 = *M. lentiflavum*; 2= *M. abscessus*; 3 = *M. avium*; 4 = *M. chelonae*; 5 = *M. fortuitum*; 6 = *M. gordonae*; 7 = *M. intracellulare*; 8 = *M. kansasii*; 9 = *M. arupense*; 10 = *M. conceptionense*; 11 = *M. peregrinum*; 12 = *M. smegmatis*; 13 = *M. chimaera*; 14 = *M. mucogenicum*; 15 = *M. yongonense*; 16 = *M. simiae*; 17 = *Mycobacterium* spp; 18 = *M. canariense*; 19 = *M. avium* & *M. intracellulare*; 20 = *M. chelonae* & *M. gordonae*; 21 = *M. avium* & *M. chelonae*; 22 = *M. fortuitum* & *M. chelonae*).



3.2 Cluster Analysis – Factor Analysis

The studied parameters of the data set were:

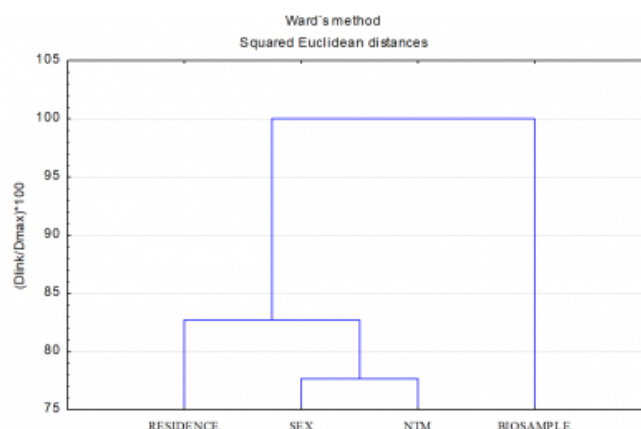
- The “Sex” of the patient
- The “Biosample” the kind of biological sample from positive NTM patients
- The “Residence” of positive NTM patients
- The “NTM” that are the species of nontuberculous mycobacteria found in patients

3.2.1 Implementation of CA and FA to all data set

CA is conducted to all data set and in that case the dendrogram presented in Figure 5 is produced.

Figure 5

Hierarchical dendrogram for the five studied parameters of all positive NTM patients.



The produced dendrogram grouped the studied parameters into two main clusters, as follows:

- **1st Cluster** (two parameters included): “BIOSAMPLE”.
- **2nd Cluster** (three parameters included): “RESIDENCE”, “SEX” and “NTM”.

Consequently, CA results indicated that the parameters “NTM”, “SEX” and “RESIDENCE” correlated to each other.

Then FA is conducted to the whole data set and in Table 1(a, b) the loadings of the extracted new VFs of unrotated and varimax normalized factors are included. Finally, according to screeplot criterion, three eigenvalues are considered significant.

Non-Tuberculous Mycobacteria As Environmental Risk Factors For Human Infectious Diseases – Isolation Of Non-Tuberculous Mycobacteria From Patients In The Thessaly Region (Central Greece) And Their Correlation With Sarcoidosis Disease

Table 1

Factor Loadings (Un = Unrotated and Var = Varimax normalized), Eigenvalues, and Total variance (%).

(a) When two (2) extracted Factors

Parameter	VF1 (Un)	VF1 (Var)	VF2 (Un)	VF2 (Var)
SEX	0,728629	0,580373	0,114085	-0,455062
BIOSAMPLE	-0,375409	0,079466	-0,834582	0,911671
NTM	0,538855	0,710430	-0,492045	0,166624
RESIDENCE	0,527584	0,581948	-0,248858	-0,040160
Eigenvalue	1,240541		1,013581	
Total (Cumulative) variance (%)	31,01		25,34 (56,35)	

(b) When three (3) extracted Factors

Parameter	VF1 (Un)	VF1 (Var)	VF2 (Un)	VF2 (Var)	VF3 (Un)	VF3 (Var)
SEX	0,728629	0,510803	0,114085	-0,476947	0,119742	0,264300
BIOSAMPLE	-0,375409	0,081910	-0,834582	0,912633	-0,075280	0,059314
NTM	0,538855	0,907172	-0,492045	0,104237	0,550605	-0,042567
RESIDENCE	0,527584	0,018748	-0,248858	0,009965	-0,781305	0,974814
Eigenvalue	1,240541		1,013581		0,933608	
Total (Cumulative) variance (%)	31,01		25,34 (56,35)		23,34 (79,69)	

Data of Table 1 shows that there is a strong moderating correlation of the parameters “SEX”, “NTM” and “RESIDENCE” with the Factor 1 and this is in full agreement with the results of CA.

3.3 Cluster Analysis (K-Means Clustering)

The goal of the k-means algorithm of CA is to find the optimum “partition” for dividing a number of objects into k clusters. This procedure moves objects around from cluster to cluster with the goal of minimizing the within-cluster variance and maximizing the between-cluster variance.

CA (K-Means Clustering) for all data set was conducted to find the most significant parameters and the number of the formed clusters. The number of formed clusters and their parameters are presented below:

- **1st Cluster: “SEX”.**
- **2nd Cluster: “BIOSAMPLE”, “NTM” and “RESIDENCE”**

The data above shows that 2 clusters were formed; the 1st cluster included the parameter “SEX” while the 2nd cluster included the parameters “BIOSAMPLE”, “NTM” and “RESIDENCE”.

3.4 Principal Components and Classification Analysis

Based on Cluster Analysis (K-Means Clustering) results, Principal Components and Classification Analysis for all data set were performed. All the parameters were considered

active parameters. Table 2 presents the factor loadings and the eigenvalues of the extracted factors, which explained the 100.0% of total variation.

Table 2

Factor coordinates of the variables (Factor-variable correlations (factor loadings)).

Variable	Factor 1	Factor 2	Factor 3	Factor 4
SEX	0,726332	0,122388	0,131156	-0,663521
RESIDENCE	0,529818	-0,254384	-0,782617	0,205166
BIOSAMPLE	-0,366676	-0,840887	-0,053427	-0,394465
NTM	0,550038	-0,477149	0,545036	0,415599
Eigenvalues	1,24526	1,01445	0,929609	0,810679
Total variance (%)	31,13	25,36	23,24	20,27
Cumulative Total variance (%)	31.1	56.5	79.7	100.0

Using the data of Table 2, it could shown that Factor 1 included three variables (“SEX”, “RESIDENCE” and “NTM”) which correlated moderately to each other. The second extracted factor included the variable “BIOSAMPLE” that correlated weakly to moderately with “NTM”; the variables “RESIDENCE” and “NTM” correlated moderately to each other in third factor, and lastly, in the fourth factor they showed a moderately to weakly correlation between the variables “SEX” and “NTM”. Additionally, the contribution of each variable in the extracted factors is presented in Table 3.

Table 3

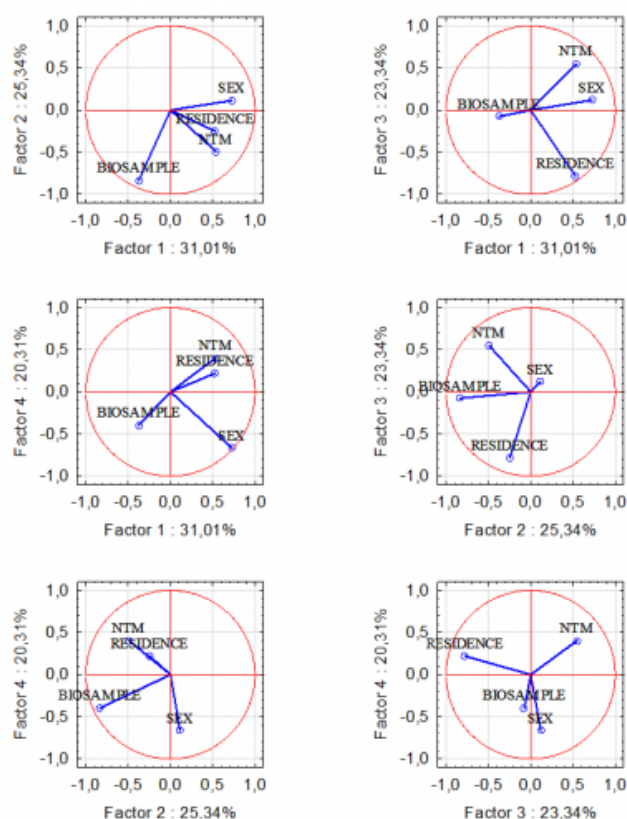
Variable contributions per extracted Factor.

Variable	Factor 1	Factor 2	Factor 3	Factor 4
SEX	0,423654	0,014765	0,018505	0,543076
RESIDENCE	0,225420	0,063789	0,658867	0,051923
BIOSAMPLE	0,107971	0,697017	0,003071	0,191941
NTM	0,242955	0,224428	0,319558	0,213059

Data of Table 4 shows that the variables “SEX”, “RESIDENCE” and “NTM” mainly contributed in Factor 1; the variable “BIOSAMPLE” with “NTM” in Factor 2; Factor 3 contributed from variables “RESIDENCE” and “NTM”; and the contributed variables in Factor 4 are “SEX” and “NTM”. Figure 6 shows how the studied variables of the extracted factors were located in the space.

Figure 6

Projection of the studied variables on the factor-plane.



In the above graphical presentation (Figure 6) when the variables were located near each other in space it meant that there is a correlation among them. Thus, when we see the plots of Factor 1 vs Factor 2, Factor 1 vs Factor 4 and Factor 2 vs Factor 4, the variables “NTM” and “RESIDENCE” are plotted nearly in space, showing that these variables were correlated to each other.

DISCUSSION

Only a few studies have attempted to look into the impact of nontuberculous mycobacteria on human’s health and specifically in Greece there are very few. Research studies in the city of Patra for the period 1998 to 2000 (Peloponnese) showed that the types of nontuberculous mycobacteria isolates in patients were *M. flavescens*, *M. gordonae*, *M. chelonae* and *M. Terrae*; research studies in central Greece for the period 2004 to 2006 showed that the NTM species most commonly isolated in patients from this area were *M. fortuitum* (51.6%) and *M. peregrinum* (34.5%). In the study carried out in Crete during the period from 2000 to 2009, the most common NTM species that were isolated from patients were the MAC complex (*M. avium* and *M. intracellulare*)

(8.6%) and *M. kansasii* (3.4%). In research study carried out in Athens during the period from 2007 to 2013, *M. avium* (13%), *M. intracellulare* (10%), *M. gordonae* (14%) and *M. fortuitum* (12%) were isolated. Finally, in a study carried out in the region of Larissa, the most common NTM species that were isolated were the *M. fortuitum* (30.8%), *M. gordonae* (22.7%) and *M. peregrinum* (12%), while the percentage of *M. avium* and *M. intracellulare* was 2.1% and 1.8%, respectively [Vantarakis et al. 1998; Tsintzou et al. 2000; Gerogianni et al. 2008; Gitti et al. 2011; Panagiotou et al. 2014; Dovriki et al. 2016].

From the Figure 1 it can be seen that positive NTM patients are dispersed widely in the Thessaly region and this is in full agreement with the literature. Moreover, the distribution of NTM species worldwide varies by geographic regions. Recent studies in the USA, Japan and other counties worldwide show that the most frequently reported NTM species that mainly affect middle aged or elderly patients are *M. avium*, *M. fortuitum* and *M. kansasii* [Gerogianni et al. 2008]. These differences in results are mainly due to the different methods used for the determination and identification of NTM species as well as the different composition of the population of studied patients, and finally the regional variations in environmental conditions.

A total of 296 patients (60% male) of the University Hospital of Larissa were tested positive for NTM during the period 2010 to 2016. The permanent residence of these patients was the region of Thessaly (central Greece) and specifically 18 geographical locations there (Figure 1). The majority of the positive NTM patients lived in the cities, while the rest was distributed over the whole areas of the Thessaly region (Figure 1).

As it is shown in Figure 2, the biological samples that were studied came mainly from the pulmonary (53%) and pathological (30%) clinic of the mentioned hospital. This is in line with other studies which report that the most common source of biological samples taken to isolate NTM is the pulmonary clinic [Archibald and Jarvis 2011; Billinger et al. 2009; Epsom and Winthrop 2012; Gerogianni et al. 2008; Moore et al. 2010; Iseman and Marras 2008; Lyu et al. 2011; Maekawa et al. 2011; Panagiotou et al. 2014; Primm et al 2004; Chou et al. 2014; Dovriki et al. 2016].

Overall, for the aforementioned set term, the nontuberculous mycobacteria identified were *M. fortuitum* (36%), *M. gordonae* (21%), *M. chelonae* (15%); *M. intracellulare* (9%),

M. avium (5%) and *M. abscessus* (4%). It is noteworthy that *M. tuberculosis* was isolated in one patient and in six patients we isolated simultaneously 2 NTM species (Figure 3). According to the scientific literature, the presence of NTM is the cause for the false (positive or negative) diagnosis of tuberculosis [Gerogianni et al. 2008].

Recently, the study of possible correlation between the presence of NTM in humans and sarcoidosis disease became of great interest. Thus, there is research data arguing that a correlation between NTM and sarcoidosis may exist [Kon and Du Bois 1997; Dumouchel-Champagne et al. 2009; Mortaz et al. 2014]

In the present study, the geographical distribution of sarcoidosis cases in the Thessaly region gives us indications that there may be a correlation between NTM and sarcoidosis disease. Additionally, multivariate analysis methods have been used to identify possible correlations between the presence of NTM in patients and other parameters concerning them. For this purpose, Box & Whisker plot analysis, Cluster Analysis, and Factor Analysis were performed.

The Box & Whisker plot analysis showed that there is correlation among the parameters “BIOSAMPLE”, “NTM” and “RESIDENCE” (Figure 4). However, there is no correlation of them with the studied parameter “SEX”.

When CA was conducted to all data sets, the produced dendrogram (Figure 5) showed that NTM isolated from patients correlated with the parameters “SEX” and “RESIDENCE” while there is no correlation with the parameter “BIOSAMPLE”. Both, the “Box & Whisker plot analysis” and the “Cluster analysis” proved that there is a correlation between the parameters “NTM” and “RESIDENCE”. Furthermore, the FA results confirmed the CA results reinforcing their validity. Concerning FA results, when the parameters located closer to each other in space, it could be concluded that they were more closely correlated to each other. Thus, Figure 6 shows that the parameters “NTM” and “RESIDENCE” are indeed correlated to each other because these parameters were located close to each other in space.

Additionally, CA (K-Means Clustering) was applied on the whole data set and two clusters were generated. The most significant parameters for this clustering were the parameters “BIOSAMPLE”, “NTM” and “RESIDENCE”, grouped

together into the second cluster, indicating that there is a correlation among them.

Subsequently, Principal Component Analysis (PCA) and Classification Analysis were performed to the whole data set. Three new factors were extracted which explained the 79.7% of the total variance (Table 2). Specifically, the factor coordinates of the parameters “NTM” and “RESIDENCE” are around 0.50, thus concluding to the fact that they are moderately interrelated. Finally, this interrelation is shown in Figure 6 where the parameters are close to each other in the space, indicating the existence of their interrelation. The results show a moderate correlation between the parameters “NTM” and “RESIDENCE” and this fully comply with the results of CA Analysis and FA Analysis.

The results of the present study support the argument that NTM are widely dispersed in the environment. Moreover, there is evidence that the presence of NTM in patients may be correlated with sarcoidosis disease. However, because of the differences observed in the results of the relevant studies, further studies are needed to determine the significance of NTM in human health in Greece and worldwide, and to find if there is a correlation of them with sarcoidosis disease.

4. CONCLUSION

Multivariate statistical methods, like Box & Whisker plot analysis, CA, and FA were used to discover possible correlations among the NTM found in patients and other parameters “SEX”, “BIOSAMPLE” and “RESIDENCE” concerning these patients. Box & Whisker plot analysis showed that there is correlation among the parameters “BIOSAMPLE”, “NTM” and “RESIDENCE”. CA results indicated that there is a correlation between the parameters “NTM” and “RESIDENCE”. Subsequently, FA was conducted on the same data set and the results of CA were confirmed by the FA results. In addition, CA (K-Means Clustering) was applied to the same data set confirming the correlation between these parameters once again. Furthermore, based on the results of CA (K-means clustering), Principal Components and Classification Analysis we were able to show showed that the two above mentioned parameters are indeed correlated.

Consequently, these three multivariate statistical methods (i.e. Box & Whisker plot analysis, Cluster Analysis and Factor Analysis) have shown that the “NTM” found in patients and the “RESIDENCE” of these patients correlate to

each other and proved that NTM are widely dispersed in the environment. In addition, based on the geographical distribution of NTM and sarcoidosis cases in the region of Thessaly, we can assume that there is evidence of a possible correlation between them.

References

1. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 1999 Aug;160(2):736-55.
2. Ainslie GM, Benatar SR. "Serum angiotensin converting enzyme in sarcoidosis: sensitivity and specificity in diagnosis: correlations with disease activity, duration, extra-thoracic involvement, radiographic type and therapy". *Quarterly J Med* 1985;55:253-270.
3. Archibald, L. K., Jarvis, W. R. (2011). Health Care-Associated Infection Outbreak Investigations by the Centers for Disease Control and Prevention, 1946–2005. *Am J Epidemiol.*, 174:S47–S64.
4. Billinger, M. E., Olivier, K. N., Viboud, C., Montes de Oca, R., Steiner, C., Holland, S. M., Prevots, D. R. (2009). Nontuberculous Mycobacteria-associated Lung Disease in Hospitalized Persons, United States, 1998–2005. *Emerging Infectious Diseases*, 15 (10):1562-1569.
5. Blankstein R, Osborne M, Naya M, et al. "Cardiac positron emission tomography enhances prognostic assessments of patients with suspected cardiac sarcoidosis". *J Am Coll Cardiol* 2014;63:329-336.
6. Chou, M. P., Clements, A. C. A., Thomson, R. M. (2014). A spatial epidemiological analysis of nontuberculous mycobacteria infections in Queensland, Australia. *BMC Infectious Diseases*, 14:279.
7. Costabel U, Bonella F, Ohshimo S, et al. "Diagnostic modalities in sarcoidosis: BAL, EBUS, and PET". *Semin Respir Crit Care Med* 2010 Aug;31(4):404-8.
8. Cremers JP, Van Kroonenburgh MJ, Mostard RL, et al. "Extend of disease activity assessed by 18F-FDG-PET/CT in a Dutch sarcoidosis population". *Sarcoidosis Vasc Diffuse Lung Dis* 2014;31:37-45.
9. Dantec, C. L., Duguët, J. P., Montiel, A., Dumoutier, N., Dubrou, S., Vincent, V. (2002). Occurrence of mycobacteria in water treatment lines and in water distribution systems. *Applied and Environmental Microbiology*, 68(11):5318-5325.
10. Dovriki E., Gerogianni I., Petinaki E., Hadjichristodoulou Ch., Papaioannou A., Gourgoulisanis K. (2016). Isolation and Identification of Non Tuberculous Mycobacteria from Hospitalized Patients and Drinking Water Samples – Examination of their Correlation by Chemometrics. *Environmental Monitoring and Assessment*, 188(4): 188-247.
11. Drent M, Loxer EE, De Vries J. "Sarcoidosis-associated fatigue". *Eur Respir J* 2012;40:255-263.
12. Dumouchel-Champagne H, Charlier-Woerther HC, Boibieux A, Ffrench M, Carret G, Chidiac C, Peyramond D, Ader F. Disseminated nontuberculous infections with *Mycobacterium genavense* during sarcoidosis. *European Respiratory Review* 2009 18: 299-301; DOI: 10.1183/09059180.00002209.
13. Epton, E., Winthrop, K. (2012). Nontuberculous Mycobacterial Lung Disease: An Emerging Disease in the Elderly. *Open Longevity Science*, 6:92-100.
14. Falkinham III, J. O. (2009). Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. *Journal of Applied Microbiology*, 107:356–367.
15. Falkinham III, J. O. (2011). Nontuberculous Mycobacteria from Household Plumbing of Patients with Nontuberculous Mycobacteria Disease *Emerging Infectious Diseases*, 17 (3):419-424.
16. Gerogianni, I., Papala, M., Kostikas, K., Petinaki, E., Gourgoulisanis, K. I. (2008). Epidemiology and clinical significance of mycobacterial respiratory infections in Central Greece. *Int J Tuberc Lung Dis*, 12(7):807–812.
17. Gill, C. O., Saucier, L., Meadus, W. J. (2011). *Mycobacterium avium* subsp. *Paratuberculosis* in dairy products, meat, and drinking water. *Journal of Food Protection*, 74 (3):480-499.
18. Gitti, Z., Mantarakis, E., Maraki, S., Samonis, G. (2011). Clinical significance and antibiotic susceptibilities of nontuberculous mycobacteria from patients in Crete, Greece. *Future Microbiol.*, 6(9):1099-1109.
19. Griffith, D. E., Aksamit, T., Brown – Elliott, B. A., Catanzaro, A., Daley, C., Gordin, F., et al. (2007). An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med*, 175:367-416.
20. Gupta D, Dadhwal DS, Agarwal R, et al. "Endobronchial ultrasound-guided transbronchial needle aspiration vs conventional transbronchial needle aspiration in the diagnosis of sarcoidosis". *Chest* 2014;146:547-556.
21. Iannuzzi MC, Fontana JR. "Sarcoidosis: clinical presentation, immunopathogenesis, and therapeutics". *JAMA* 2011;26:305(4):391-9.
22. Iseman, M. D., Marras, T. K. (2008). The Importance of Nontuberculous Mycobacterial Lung Disease. *Am J Respir Crit Care Med*, 178:999–1001.
23. James D. "Sarcoidosis of the skin". *Semin. Respir. Med* 1992 13:422–441.
24. James DG. "Ocular sarcoidosis". *Ann N Y Acad Sci* 1986;465:551-63.
25. Jolobe, O. M. P. (2011). Atypical tuberculous pleural effusions. *European Journal of Internal Medicine*, 22:456–459.
26. Kaevska, M., Hruska, K. (2010). Research on *Mycobacterium avium* during the period 1995 to 2009. *Veterinari Medicina*, 55 (10):473-482.
27. Kankya, C., Muwonge, A., Djonne, B., Munyeme, M., Opuda-Asibo, J., Skjerve, E., Oloya, J., Edvardsen, V., Johansen, T. B. (2011). Isolation of non-tuberculous mycobacteria from pastoral ecosystems of Uganda: Public Health significance. *BMC Public Health*, 11:320.
28. Keijsers RG, Verzijlbergen FJ, Oyen WJ, et al. "18F-FDG PET, genotype-corrected ACE and sIL-2R in newly diagnosed sarcoidosis". *Eur J Nucl Med Mol Imaging* 2009;36:1131-1137.
29. Kon OM, du Bois RM. Mycobacteria and sarcoidosis. *Thorax* 1997;52(Suppl 3):S47–S51.
30. Lyu, J., Jang, H. J., Song, J. W., Choi, C. M., Oh, Y. M., Lee, S. D., Kim, W. S., Kim, D. S., Shim, T. S. (2011). Outcomes in patients with *Mycobacterium abscessus* pulmonary disease treated with long-term injectable drugs. *Respiratory Medicine*, 105:781-787.
31. Maekawa, K., Ito, Y., Hirai, T., Kubo, T., Imai, S., Tatsumi, S., Fujita, K., Takakura, S., Niimi, A., Iinuma, Y., Ichiyama, S., Togashi, K., Mishima, M. (2011).

Environmental Risk Factors for Pulmonary Mycobacterium avium-intracellulare Complex Disease. CHEST, 140 (3):723-729.

32. Massart, D. L., Kaufman, L. (1983). The Interpretation of Chemical Data by the Use of Cluster Analysis. J. Wiley, New York.
33. Moore, J. E., Kruijshaar, M. E., Ormerod, L., Drobniewski, F., Abubakar, I. (2010). Increasing reports of non-tuberculous mycobacteria in England, Wales and Northern Ireland, 1995-2006. BMC Public Health, 10:612.
34. Moorthy, R. S., Valluri, S., Rao, N. A. (2012). Nontuberculous Mycobacterial Ocular and Adnexal Infections. Surv Ophthalmol, 57 (3):202 – 235.
35. Mortaz E, Adcock IM, Barnes PJ. Sarcoidosis: Role of non-tuberculosis mycobacteria and Mycobacterium tuberculosis. Int J Mycobacteriol. 2014 Dec;3(4):225-9. doi: 10.1016/j.ijmyco.2014.10.008.
36. Ness, R., Kip, K., Hillier, S., Soper, D., Stamm, C., Sweet, R., Rice, P., Richter, H. (2005). A Cluster Analysis of Bacterial Vaginosis-associated Microflora and Pelvic Inflammatory Disease. American Journal of Epidemiology, 162:585-590.
37. Nguyen, H., Altinger, J., Carrieri-Kohlman, V., Gormley, J., Paul, S., Stulberg, M. (2003). Factor Analysis of Laboratory and Clinical Measurements of Dyspnea in Patients with Chronic Obstructive Pulmonary Disease. Journal of Pain and Symptom Management, 25:118-127.
38. Oksanen V. "Neurosarcoidosis". Sarcoidosis 1994 Mar;11(1):76-9.
39. Olive KE, Kataria YP. "Cutaneous manifestations of sarcoidosis". Arch Int Med 1985;145:1811–1814.
40. Panagiotou, M., Papaioannou, A. I., Kostikas, K., Paraskeua, M., Velentza, E., Kanellopoulou, M., Filaditaki, V., Karagiannidis, N. (2014). The Epidemiology of Pulmonary Nontuberculous Mycobacteria: Data from a General Hospital in Athens, Greece, 2007–2013. Pulmonary Medicine, 2014: 894976. Doi: 10.1155/2014/894976.
41. Papaioannou, A., Karamanis, G., Rigas, I., Spanos, T., Roupa, Z. (2009). Determination and Modelling Clinical Laboratory Data of Healthy Individuals and End-Stage Renal Failure Patients. Cent. Eur. J. Med., 4(1):37-48.
42. Penn, R., Steehler, M. K., Sokohl, A., Harley, E. H. (2011). Nontuberculous mycobacterial cervicofacial lymphadenitis - A review and proposed classification system. International Journal of Pediatric Otorhinolaryngology, 75:1599–1603.
43. Poupard, J., Gagnon, B., Stanhope, M., Stewart, C. (2002). Methods for Data Mining from Large multinational Surveillance Studies. Antimicrobial Agents and Chemotherapy, 46:2409-2419.
44. Primm, T. P., Lucero, CA., Falkinham III, J. O. (2004). Health impacts of environment mycobacteria. Clinical Microbiology Reviews, 17(1):98-106.
45. Roux, A. L., Catherinot, E., Ripoll, F., Soismier, N., Macheras, E., Ravilly, S., et al. (2009). Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in France. Journal of Clinical Microbiology, 47(12):4124-4128.
46. Russell, C. D., Claxton, P., Doig, C., Seagar, A. L., Rayner, A., Laurenson, I. F. (2014). Non-tuberculous mycobacteria: a retrospective review of Scottish isolates from 2000 to 2010. Thorax, 69:593-595.
47. Sharma OP. "Cardiac and neurologic dysfunction in sarcoidosis". Clin Chest Med 1997;18:813–825.
48. Sobic-Saranovic DP, Grozdic IT, Videnovic-Ivanof J, et al. "Responsiveness of FDG-PET/CT to treatment of patients with active chronic sarcoidosis". Clin Nucl Med 2013;38:516-521.
49. Tavee J, Culver D. "Sarcoidosis and small-fiber neuropathy". Curr Pain Headache Rep 2011;15:201-206.
50. Tsintzou, A., Vantarakis, A., Pagonopoulou, O., Athanassiadou, A., Papapetropoulou, M. (2000). Environmental mycobacteria in drinking water before and after replacement of the water distribution network. Water, Air, and Soil Pollution, 120:273-282.
51. Vanderginste, B., Massart, D. L., Buydens, L., De Jong, S., Lewi, P., Smeyers-Verbeke, J. (1998). Handbook of Chemometrics and Qualimetrics. Elsevier, Amsterdam.
52. Vantarakis, A., Tsintzou, A., Diamandopoulos, A., Papapetropoulou, M. (1998). Non-tuberculous mycobacteria in hospital water supplies. Water, Air, and Soil Pollution, 104:331-337.
53. Vogt, W., Nagel, D. (1992). Cluster Analysis in Diagnosis. Clin. Chem., 38:182-198.

Author Information

Eleni Dovriki

Department of Respiratory Medicine, Medical School, University of Thessaly
Larissa, Greece

Fotini Bardaka

Department of Respiratory Medicine, Medical School, University of Thessaly
Larissa, Greece

Irini Gerogianni

Department of Respiratory Medicine, Medical School, University of Thessaly
Larissa, Greece

Efi Petinaki

Department of Microbiology, Medical School, University of Thessaly
Larissa, Greece

Kostas I. Gourgoulidis

Department of Respiratory Medicine, Medical School, University of Thessaly
Larissa, Greece

Agelos Papaioannou

Department of Medical Laboratories, Technological Education Institution of Thessaly
Larissa, Greece