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Short Communication

The Prevalence of *Mycobacterium tuberculosis* Complex Species in TB Patients Living in Urban Setting in Central Poland

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Abstract

The *Mycobacterium tuberculosis* complex (MTBC) consists of species and subspecies that cause tuberculosis (TB) in humans and animals. As all MTBC strains are closely related at the molecular level, the exact distinction between them is not implemented in routine diagnostic techniques but could be used for tracking the zoonotic infection route, identification of drug resistance and epidemiological profiling. This study aimed to analyse the distribution of MTBC species in TB patients in the urban setting of Warsaw (central Poland). A total of 58 clinical isolates derived from patients diagnosed with TB were analysed. All specimens were processed for culture, DNA extraction and PCR. The distribution of species was investigated using GenoType MTBC molecular assay. All TB cases analysed were caused by *Mycobacterium tuberculosis*. *M. tuberculosis* appears to be the predominant cause of TB in Warsaw, central Poland, as no cases found of human TB caused by *M. bovis* or other MTBC strains were found.

Keywords: Genotyping, *Mycobacterium tuberculosis* complex, *Mycobacterium bovis,* Tuberculosis

Introduction

Tuberculosis (TB) remains a significant health threat in the world. In 2020, it was the 13th leading cause of death, killing a total of 1.5 million people [1]. In Poland, the reported incidence of this communicable disease was 13.9 cases per 100 000 in 2019 [2], still too many to name Poland a low-incidence country according to the Word Health Organization (WHO) definition (less than 10 cases per 100 000) [3]. TB is primarily caused by the *Mycobacterium tuberculosis* complex (MTBC) in humans [3]. MTBC comprises various species and subspecies which are adapted to humans (*M. africanum, M. canetti and M. tuberculosis*) as well as strains adapted mainly to animals (i.e. *M. bovis*, *M. caprae)*. These species share an astonishing 99.95% sequence homology, including the ribosomal RNA genes [4]. For this reason, routine identification carried out by a microbiology laboratory reveals mycobacteria only at the complex level [5]. Despite their similarities, MTBC species differ epidemiologically as well as in host range, pathogenicity, geographic distribution, and drug resistance [6]. For example, *M. bovis* can be transmitted to humans from a wide variety of hosts,

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including domestic and wild animals [7]. Bovine TB is spread primarily from animal to animal via respiratory secretion (e.g. in livestock trade) or by food contaminated with *M. bovis* [8, 9]. Due to the scarce data on the actual distribution of MTBC species among patients in Poland, our study aimed to examine the prevalence of MTBC species in clinical samples using GenoType MTBC molecular assay (Hain Lifescience GmbH, Germany) obtained from patients between 2017 and 2020 in the urban setting of Warsaw, central Poland.

Materials and Methods

General Study Design

This was a retrospective and descriptive study based on data from 58 patients (38 men and 20 women) who were diagnosed with TB and hospitalized in the University Clinical Centre of the Medical University of Warsaw, Poland between 2017 and 2020. We enrolled all patients for whom at least one MTBC member isolate had been cultured. The study protocol (Molecular identification of *Mycobacteria* spp. within *Mycobacterium tuberculosis* complex in Polish population) was accepted by the Institutional Review Board (AKBE/57/2021).

A set of 58 MTBC strains isolated from pulmonary or extrapulmonary specimen from 2017 to 2020 were analysed (sputum – 27; bronchoalveolar lavage fluid – 23; pleural fluid – 4; lung biopsy – 1, gastric biopsy – 1 and tracheal aspirate – 2). Only one specimen per patient was used in the analysis. Samples were homogenized and decontaminated using standard methods (4% NAOH, 2.9% sodium citrate, 0.5% N-acetyl-L-cysteine). Specimen were concentrated up to 1-1.5 ml, and examined microscopically with the Ziehl-Neelsen staining. Samples were inoculated on two solid media (Löwenstein-Jensen and Stonebrink) and liquid medium Middelbrook 7H9 in automated system (Bactec MIGIT, BD, USA). Then incubated at 37ºC for up to 8-10 weeks for solid and up to 6 weeks for liquid media.

DNA Extraction

DNA extraction from cultured material was performed with GenoLyse Kit (Hain Lifescience GmbH, Germany) from solid and liquid media according to the manufacturer's instruction. Briefly, bacteria grown in a liquid medium were centrifuged (9700 rpm for 15 min), fluid was removed from the sample and cell pellet was prepared. In the next step, lysis under alkaline conditions (100 µl of lysis buffer containing <1% non-ionic surfactant and <0.2% NaOH) and at high temperature (5 min at 95ºC in a water bath) was performed. After a short centrifugation, 100 µl of neutralization buffer was added. The tube was vortexed and centrifuged at 3000 rpm for 5 min. Five microliters of the supernatant were used for subsequent amplification.

Amplification

PCR was performed with 1U Platinum Taq polymerase and buffers (Thermo Fisher Scientific, Invitrogen, USA), with primers detecting DNA from *M. africanum*, *M. bovis* Bacillus Calmette-Guérin (BCG), *M. bovis*, *M. caprae*, *M. microti* and *M. tuberculosis*/*M. canetti*.

Strain Typing

To investigate the prevalence of MTBC species we used GenoType MTBC molecular assay (Hain Lifescience GmbH, Germany) according to the manufacturer's instructions.

Results and Discussion

This report assesses the prevalence of *Mycobacterium tuberculosis* complex species within the representative group of patients with TB in a big city in the centre of Poland. Our cohort comprised mainly patients of Polish (49) and Ukrainian (3) nationality, with single cases from India, Nepal, Vietnam, Indonesia and Guinea. All patients diagnosed with TB lived in Poland for at least 5 years. More data on the characteristics of the group are shown in Table 1. The prevalence of extrapulmonary tuberculosis in our cohort is comperable to general Polish population [2], however, it varies depending on the region [3, 10]. All isolates obtained from TB patients were identified as *M. tuberculosis/M. canettii* (n = 58). We found no cases of zoonotic TB caused by *M. bovis* infection or other subspecies of MTBC.

These results correspond to previous research by Safianowska et al., who differentiated MTBC strains in a similar cohort of 161 TB patients in the same region of Poland with the same results. Similarly, to their data, we found no cases of zoonotic TB caused by *M. bovis* infection or other subspecies of MTBC. *M. bovis* has a wide host range, infecting wildlife (bison, badgers, deer, etc.) and farmed animals like cattle and pigs. In Poland, bovine TB cases are reported and controlled by the General Veterinary Inspectorate. Despite Poland gaining bovine TB-free status in 2009, some cases of animal TB have still been reported in the literature [11]. We found no cases of TB caused by pathogens other than *M. tuberculosis/M. canettii* in our isolates. Despite all of this, there are still chances that *M. bovis* has some prevalence in the Polish population. The epidemiological investigation described the transmission of bovine tuberculosis in cattle, where a human could be a vector for *M. bovis*. [12]. Recently, a paper on the first human infection with *M. caprae* in Poland was published

Table 1. Characteristics of the cohort group.

Characteristics, *n* = 58	
Males/Females (%)	38/20 (65.5/34.5)
Age (years)	55±18
Socioeconomic status	
High (%)	19 (32.8)
Middle (%)	6 (10.3)
Poor (%)	14 (24.1)
Unknown (%)	19 (32.8)
Homelessness (%)	14 (24.1)
Case Definition	
New case (%)	46 (79.3)
Relapse (%)	4 (6.9)
Previous failure (%)	2 (3.4)
Treated previously (%)	1 (1.7)
Unknown status (%)	5 (8.6)
Anaemia	
Haemoglobin (g/dl)	12.1±3.9
Type of anaemia	
Normocytic (%)	24 (43.6)
Microcytic (%)	11 (20.0)
Macrocytic (%)	6 (10.9)

Comorbidities	
Alcohol use (%)	23 (39.7)
Unknown status of alcohol use (%)	19 (32.8)
Malignancy (%)	5 (8.6)
Chronic lung disease (%)	4 (6.9)
Autoimmune disease (%)	2 (3.4)
Immunosuppressive drugs (%)	2 (3.4)
Chronic liver disease (%)	1 (1.7)
Symptoms preceding the diagnosis	
Cough (%)	19 (32.8)
Febrile sense (%)	17 (29.3)
Dyspnoea (%)	15 (25.9)
Sputum expectoration (%)	13 (22.4)
Weight loss (%)	13 (22.4)
Fatigue (%)	12 (20.7)
Chest discomfort (%)	6 (10.3)
Night sweats (%)	6 (10.3)
Site of tuberculosis	
Pulmonary (%)	55 (94.8)
Extrapulmonary (%)	2 (3.4)
Both pulmonary and extrapulmonary (%)	1 (1.7)

The data are presented as mean ± SD or number of cases and the percentages.

in 2020 [13]. To our best knowledge, no cases of TB caused by *M. canettii* have been reported in humans or animals in Poland.

Due to the low number of patients in our sample, the comparison between our results and other studies or reports from other European countries is difficult. According to the literature, *M. bovis* in humans is estimated to be responsible for a small (0.5%-7.2%) proportion of diagnosed cases of TB in developed countries [14]. Throughout 2006-2014, the United Kingdom reported a slight increase in *M. bovis* infection cases from 0.4% to 0.9% [15]. In the Netherlands, 1.4% of *M. bovis* cases were reported during 1993-2007 [16]. In 2011, Bayraktar et al. reported that in Turkey during 2007-2010 94.1% of tuberculosis was caused by *M. tuberculosis*, 4.3% by *M. bovis* and 1.6% by *M. caprae* [17].

Genotype MTBC molecular assay (Hain Lifescience GmbH, Germany) is an established method of differentiating between the MTBC species within the complex. Richter et al. in 2004 evaluated the usefulness of this assay in cultures obtained from clinical specimens. Of 75 positive samples for MTBC, 3 (4.0%) samples derived from humans were typed as *M. bovis* and 1 (1.3%) as *M. africanum* [18]. Similar research was conducted by Neonakis et al. in Greece [19]. Both articles confirmed the full concordance of the assay with the results obtained via other techniques used by respective researchers.

This study addresses a quite rare subject of the prevalence of MTBC species in a specific area of a large city in central Poland. As presented above, up-to-date data on this matter are scarce. This can lead to underestimating the possibility that patients are infected with other MTBC member than *M. tuberculosis*. Furthermore, it serves as a control of the actual spread of different MTBC strains and, in case of new species appearing, would help in discovering the infection paths and sources (e.g. animal routes). Thus, it also verifies the efficacy of the preventive measures required by law. However, there are some limitations in this study that could be addressed in the future. This study was carried out on isolates obtained from hospitalized patients in a major city, where contact with animals (especially wildlife) is not a given. Thus, our cohort lacks both in terms of size and diversity. Further multicentre research in a variety of locations involving multiple demographics would address those limitations.

The prevalence of MTBC subspecies in Poland is difficult to define due to the lack of routine in-laboratory

differentiation. As a relatively small cohort was taken into consideration in this research, further study might deliver valuable additional information useful in a wider context, e.g. capture-recapture analyses [20]. Taking into account recent reports of human TB caused by *M. bovis* or *M. caprae* in Poland and its presence in statistics in other countries, including European ones, the exact typing of MTBC isolates derived from patients within the complex could improve the treatment outcome as well as increase awareness of infection routes in the environment.

Conclusions

M. tuberculosis appears to be the predominant cause of TB in Warsaw, central Poland, as no cases of human TB caused by *M. bovis* or other MTBC strains were found.

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Ethics Approval and Consent to Participate

All procedures performed in this study followed the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This work has received approval for research ethics from the Medical University of Warsaw Review Board (AKBE/57/2021) and a proof/certificate of approval is available upon request.

Competing Interests

The authors declare no conflict of interest.

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