Transmission of Drug-Resistant Tuberculosis between Household Contacts

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Abstract
Objective: The most important measures in the control of the spread of drug-resistant tuberculosis (DR-TB) are the early detection of smear-positive patients, implementation of appropriate treatment, and tracing the chain of transmission of infection. Research on TB transmission in the environment of patients indicates that the risk of infection between close contacts, especially family members, is very high. The purpose of this study was to investigate the transmission of DR-TB within 6 family-households identified during the period of 2006-2016 in Poland.

Methods: Two PCR-based genotyping methods were used: spoligotyping, and mycobacterial interspersed repetitive unit-variable number of tandem repeats (MIRU-VNTR) typing.

Results: According to spoligotyping and MIRU-VNTR results, in all households, patients had identical Mycobacterium tuberculosis isolates, implying intra-familial transmission. Isolates from 2 families with Beijing-TB represented the pre-XDR and XDR phenotypes.

Conclusions: This study demonstrates the household setting as an important pathway of drug-resistant Mycobacterium tuberculosis transmission, and thereby reinforces the need for routine extensive screening of the housemates of TB patients.

ABBREVIATIONS
TB: Tuberculosis; IGRA: Interferon Gamma Release Assay; MIRU-VNTR: Mycobacterial Interspersed Repetitive Units-Variable Number of Tandem Repeats; DR: Drug Resistant; DS: Drug Sensitive; NTRL: National Tuberculosis Reference Laboratory; MTB: Mycobacterium Tuberculosis; AFB: Acid-Fast Bacilli; L-J: Lowenstein-Jensen; INH: Isoniazid; RMP: Rifampicin; SM: Streptomycin; EMB: Ethambutol; OFL: Ofloxacin; KAN: Kanamycin; AMK: Amikacin; CAP: Capreomycin; CTAB: Cetyltrimethyl-Ammonium Bromide; Pre-XDR: Pre-Extensively Drug-Resistant; XDR: Extensively Drug-Resistant; MDR: Multidrug-Resistant; H: Haarlem; LAM: Latin American Mediterranean; ST: Shared Type; NR: Not Registered

INTRODUCTION
Tuberculosis (TB) is an infectious disease most commonly transmitted by airborne transfer of bacteria in droplet nuclei. The most important measures in the control of the spread of the disease are the early detection of smear-positive patients, implementation of appropriate treatment, and tracing the chain of transmission of infection. Many years of research on TB transmission indicate that the risk of infection among close contacts in prisons, hospitals, schools, homeless shelters, migrants’ shelters, and other space-restricted and crowded environments, is very high [1-5]. Epidemiological investigation should therefore be extended to all persons who have contact with the TB patient, and special attention should be paid to the people living in the close proximity of patients with drug-resistant tuberculosis. Normally, the closest contacts of a positively diagnosed TB patient, including housemates, are the first to be investigated, and depending on the number of positive contacts traced in subsequent circles around the patient, this contact tracing is extended. Generally, conventional contact tracing by interview and skin and/or interferon gamma release assay (IGRA) testing of possibly exposed individuals is considered an effective strategy for identifying recent TB transmission.

However, although proximity to a smear-positive patient is a known risk factor for infection and disease, early detection of subsequent infection and disease close to TB patients remains poor in many countries. In Poland, detection has improvement recently (2012−2015), but still only 3% of cases are detected [6]. The purpose of this study was to investigate the transmission of drug-resistant TB within family-households in Poland, by using two PCR-based genotyping methods – spoligotyping, and mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR) typing [7,8].

MATERIALS AND METHODS

Patients
The study included 14 patients with pulmonary drug-resistant

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Keywords
• Tuberculosis; Drug-resistant; Household; Transmission; Genotyping
RESULTS AND DISCUSSION

It was found that, according to spoligotyping and MIRU-VNTR results, all patients within the same household had identical *Mycobacterium tuberculosis* isolates, thus implying the intra-familial transmission.

Drug susceptibility testing revealed that MTB isolates from a total of 14 patients studied were resistant at least to one antimicrobial agent tested (Table 1). In two families (A and E) with two patients each, infected family members were infected with INH-mono-resistant phenotype MTB. All isolates from family B exhibited resistance to SM. In family D, isolates from wife and husband were resistant to INH and SM. In the remaining 2 families (C and E), family members were infected with pre-XDR (pre-extensively drug-resistant) bacteria, defined as MTB resistance to at least INH and RMP, which is obligatory for multidrug resistance in addition to either a fluoroquinolone or one of the injectable aminoglycosides (AMK, KAN, or CAP), and XDR (extensively drug-resistant) phenotype, defined as resistance to INH, RMP, one of the fluoroquinolones and one of the injectable aminoglycosides [13].

Sputum smears for AFB were positive in 7 patients in 5 families (71% of all families under study). Four of them represented 'index case' status. In 2 (29%) families, all patients were smear positive, and in the remaining 1 (14%) family 2 patients were negative for AFB microscopy (Table 1).

As demonstrated in other studies, in a household with a person with active tuberculosis, 40-50% of the remaining members of the family can be infected, and approximately 5% of them will develop the active form of the disease [14-16]. Families with a history of drug resistant TB and young children are particularly at risk. Such families should be monitored closely and, according to experts, surveillance of family members and others in close proximity should last 2 years in the case of MDR-TB, and 4 years in the event of XDR-TB [14]. Adults are generally responsible for infection of children due to the fact that children always live in a household with other people, whereas adults may live alone. In our study there was a family-household where the mother (index case) was the source of the TB infection in the 12-year-old son. It is possible that both infections could have happened outside the household, and the child developed symptoms faster. However, as child transmitters were more likely to be smear and culture positive (and thereby have the potential to transmit) compared to non-transmitters, we should not assume that adults within the household are always the index case. Exposure to infectious TB may have occurred within the community, with the age of the child influencing both their risk of exposure and the likelihood of transmitting to others.

In Poland, research has focused on the transmission of drug-resistant TB as an accidental phenomenon, rather than the result of close, frequent, long lasting contact. It has been proven that close populations also transmit TB, and supply on average 30-40% of study subjects [17,18]. Although incidence rates for multidrug-resistant-TB (MDR-TB) in Poland remain stable, it is disturbing from an epidemiological point of view to identify MDR-TB forms among newly diagnosed patients, as is observed in our analysis. This may indicate, among other things, an insufficient control of transmission of MDR-TB in Poland.
Kozińska et al. (2017)

Table 1: Selected demographic and epidemiologic characteristics of 14 TB patients, members of 6 families, investigated in the study

<table>
<thead>
<tr>
<th>Family</th>
<th>City of residence</th>
<th>Ethnicity</th>
<th>Patient</th>
<th>Family member</th>
<th>Sex</th>
<th>Age</th>
<th>History of TB</th>
<th>Specimen</th>
<th>AFB smear</th>
<th>Date of isolation</th>
<th>Drug resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Warszawa</td>
<td>Polish</td>
<td>1A</td>
<td>mother*</td>
<td>F</td>
<td>36</td>
<td>No plwocina</td>
<td>+</td>
<td>05.08.2006</td>
<td>-</td>
<td>INH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2A</td>
<td>son</td>
<td>M</td>
<td>12</td>
<td>No bronchoal-veolar lavage</td>
<td>-</td>
<td>23.08.2006</td>
<td>-</td>
<td>INH</td>
</tr>
<tr>
<td>B</td>
<td>Koszalin</td>
<td>Polish</td>
<td>1B</td>
<td>daughter*</td>
<td>F</td>
<td>23</td>
<td>No sputum</td>
<td>-</td>
<td>01.03.2008</td>
<td>-</td>
<td>SM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2B</td>
<td>father</td>
<td>M</td>
<td>58</td>
<td>Yes sputum</td>
<td>-</td>
<td>01.09.2008</td>
<td>-</td>
<td>SM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3B</td>
<td>son</td>
<td>M</td>
<td>29</td>
<td>No sputum</td>
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<td>01.09.2008</td>
<td>-</td>
<td>SM</td>
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<td></td>
<td></td>
<td></td>
<td>4B</td>
<td>son</td>
<td>M</td>
<td>27</td>
<td>No sputum +++</td>
<td></td>
<td>20.10.2008</td>
<td>-</td>
<td>SM</td>
</tr>
<tr>
<td>C</td>
<td>Łódź</td>
<td>Polish</td>
<td>1C</td>
<td>daughter</td>
<td>F</td>
<td>24</td>
<td>Yes sputum</td>
<td>-</td>
<td>01.09.2016</td>
<td>-</td>
<td>INH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2C</td>
<td>mother*</td>
<td>F</td>
<td>52</td>
<td>Yes sputum</td>
<td>+</td>
<td>06.08.2008</td>
<td>(INH+RMP+SM+EMB+OFL)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Warszawa</td>
<td>Chech-nya</td>
<td>1D</td>
<td>wife*</td>
<td>F</td>
<td>28</td>
<td>No sputum</td>
<td>-</td>
<td>05.02.2009</td>
<td>(INH+SM)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2D</td>
<td>husband</td>
<td>M</td>
<td>39</td>
<td>No sputum</td>
<td>-</td>
<td>21.02.2010</td>
<td>(INH+SM)</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Radom</td>
<td>Polish</td>
<td>1E</td>
<td>sister*</td>
<td>F</td>
<td>26</td>
<td>No sputum</td>
<td>+</td>
<td>01.09.2016</td>
<td>INH</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2E</td>
<td>sister</td>
<td>F</td>
<td>26</td>
<td>No sputum</td>
<td>+</td>
<td>26.09.2016</td>
<td>INH</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Warszawa</td>
<td>Polish</td>
<td>1F</td>
<td>father in law*</td>
<td>M</td>
<td>36</td>
<td>No sputum</td>
<td>+</td>
<td>01.03.2015</td>
<td>(INH+RMP+SM+EMB+OFL+AMK+KAN+CAP)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2F</td>
<td>son in law</td>
<td>M</td>
<td>59</td>
<td>No sputum</td>
<td>+</td>
<td>04.02.2017</td>
<td>(INH+RMP+SM+EMB+OFL+AMK+KAN+CAP)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: TB: Tuberculosis; AFB: Acid-Fast Bacilli; INH: Isoniazid; RMP: Rifampicin; SM: Streptomycin; EMB: Ethambutol; OFL: Ofloxacin; KAN: Kanamycin; AMK: Amikacin; CAP: Capreomycin; Pre-XDR: Pre-extensively Drug-Resistant; XDR: Extensively Drug-Resistant

Table 2: MIRU-VNTR profiles and spoligotypes identified among Mycobacterium tuberculosis isolates evaluated in this study. Comparison with the SITVIT database and clade assignment

<table>
<thead>
<tr>
<th>Strain</th>
<th>Family</th>
<th>ST</th>
<th>Clade</th>
<th>Spoligotype description</th>
<th>Binary</th>
<th>Octal</th>
<th>MIRU-VNTR typing pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>A</td>
<td>36</td>
<td>H3</td>
<td>------------------------------</td>
<td>777737777720771</td>
<td>343635333434337</td>
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<td>A</td>
<td>36</td>
<td>H3</td>
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<tr>
<td>1B</td>
<td>B</td>
<td>803</td>
<td>LAM9</td>
<td>------------------------------</td>
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<td>LAM9</td>
<td>------------------------------</td>
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</tr>
<tr>
<td>1C</td>
<td>C</td>
<td>NR</td>
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<td>------------------------------</td>
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<tr>
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<td>------------------------------</td>
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<td>34344333443345</td>
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<td>D</td>
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<tr>
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<tr>
<td>1E</td>
<td>E</td>
<td>53</td>
<td>T1</td>
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<tr>
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<td>E</td>
<td>53</td>
<td>T1</td>
<td>------------------------------</td>
<td>777777777760771</td>
<td>33163424232355</td>
<td></td>
</tr>
<tr>
<td>1F</td>
<td>F</td>
<td>265</td>
<td>Beijing</td>
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</tr>
<tr>
<td>2F</td>
<td>F</td>
<td>265</td>
<td>Beijing</td>
<td>------------------------------</td>
<td>000000000003771</td>
<td>342743543431359</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NR: Not Registered; ST: Shared Type; MIRU-VNTR: Mycobacterial Interspersed Repetitive Units-Variable Number of Tandem Repeats
The molecular strain typing performed in this study also shed some light on the epidemiology of TB in a wider, whole
country context. Comparison of the spoligotyping results
with a worldwide database SITVIT (http://www.pasteur-
guadeloupe.fr:8081/SITVIT_ONLINE/) provided insights into the
phylogenetic diversity of the Mycobacterium tuberculosis strains
circulating in Poland. It is of note, that at the phylogenetic level
all patient isolates fell into four major clades, i.e. the Haarlem
(H) clade (2 patients from family A), T clade (2 from family E),
the Latin American Mediterranean (LAM) clade (4 members of
family B), and the Beijing clade (4 patients from families D and
E). In the remaining family (C), isolates exhibited a spoligotype
not registered (NR) in the SITVITWEB database (Table 2).
All the clades identified in this study had been reported in
Poland previously [19]. Among these, T and H are the most
prevalent European genotypes [20,21]. Members of two family-
households in the study were infected with MTBC strains of the
Beijing genotype, which has been associated with an increased
acquisition of drug resistance, enhanced virulence, and high
transmissibility [22]. This finding, together with previous reports
on the occurrence of the Beijing strains in Poland implies their
ongoing transmission within the country. Based on the results
from past studies and this one (one family where Beijing strains
was noted originated from Chechyna), it seems that the main
source of Beijing family strains in Poland is immigration over
Poland’s eastern border. It is of note that isolates from 2 families
with Beijing-TB represented a pre-XDR and XDR phenotype.
This suggests that, in accordance with previously described
observations, in Poland the Beijing genotype can be correlated
with drug-resistant phenotype [21]. Although spoligotyping
revealed some characteristics of the population structure of
Mycobacterium tuberculosis in Poland, a relatively small sample
size and the confinement of the study to only one type of setting
precluded an accurate estimation of the share of the genotypes
identified in cases of recent TB transmission in Poland.

CONCLUSION

This study demonstrates the household setting as an
important site of drug-resistant Mycobacterium tuberculosis
transmission, and thereby reinforces the need for routine,
extensive screening of close contacts of TB patients. The most
important assumption in this study was that finding at least
two patients within the same household whose MTBC isolates
had identical genotypes was indicative of the intra-familial
transmission. However, neither the source case nor the direction
of the transmission within a given family-household could be
determined with certainty. Although analysis of clinical and
demographic data would make it seem very likely that the
location of the source case was within the family-household, the
possibility that family members hosting the same Mycobacterium
tuberculosis strain could have been infected by a source outside
the household cannot excluded.

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