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DRUG DISCOVERY AND RESISTANCE

Characteristics of drug-resistant tuberculosis in Abkhazia (Georgia), a high-prevalence area in Eastern Europe

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SUMMARY

Although multidrug-resistant (MDR) tuberculosis (TB) is a major public health problem in Eastern Europe, the factors contributing to emergence, spread and containment of MDR-TB are not well defined. Here, we analysed the characteristics of drug-resistant TB in a cross-sectional study in Abkhazia (Georgia) between 2003 and 2005, where standard short-course chemotherapy is supplemented with individualized drug-resistance therapy. Drug susceptibility testing (DST) and molecular typing were carried out for *Mycobacterium tuberculosis* complex strains from consecutive smear-positive TB patients. Out of 366 patients, 60.4% were resistant to any first-line drugs and 21% had MDR-TB. Overall, 25% of all strains belong to the Beijing genotype, which was found to be strongly associated with the risk of MDR-TB (OR 25.9, 95% CI 10.2–66.0) and transmission (OR 2.8, 95% CI 1.6–5.0). One dominant MDR Beijing clone represents 23% of all MDR-TB cases. The level of MDR-TB did not decline during the study period, coinciding with increasing levels of MDR Beijing strains among previously treated cases.
Standard chemotherapy plus individualized drug-resistance therapy, guided by conventional DST, might be not sufficient to control MDR-TB in Eastern Europe in light of the spread of "highly transmissible" MDR Beijing strains circulating in the community.

1. Introduction

Drug-resistant tuberculosis (TB) is a major public health problem, particularly in countries of the former Soviet Union affected by economic decline and failing health infrastructures.[1–4] Globally, some of these countries show the highest frequency of isolation of *Mycobacterium tuberculosis* complex (MTBC) strains of multidrug-resistant (MDR)-TB, defined as resistance to at least isoniazid and rifampicin. MDR-TB rates of up to 14% have been documented, making successful treatment of the disease difficult.[5,6] Inadequate treatment is the primary cause of acquired drug resistance. In most limited-resource countries, MDR-TB cases are identified when patients fail to respond to first-line TB therapy.[4] Prolonged periods of sputum smear positivity might then result in enhanced transmission of MDR strains, further accelerating the increase of MDR-TB incidence.[4] Consequently in countries with high prevalence of resistance, in addition to standard short-course chemotherapy, rapid diagnosis of resistance and appropriate treatment regimens are key components for the control of TB.[3]

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Interestingly, high levels and spread of MDR-TB in Eastern Europe have been found to be associated with a specific phylogenetic lineage/genotype of the MTBC, the Beijing genotype, indicating that special properties of this pathogen might also contribute to the problem of drug resistance.[7] Studies have confirmed that patients infected with Beijing genotype strains have a higher risk of MDR-TB.[3,7,8] Furthermore, Beijing strains may also contribute to the transmission of MDR-TB.[8,9] However, studies on the consequences of Beijing genotype infection for both TB transmission and drug-resistance in high-incidence regions are only sparsely available.

Here, we present the results of a cross-sectional survey, which assessed the characteristics of drug-resistant TB in Abkhazia (Georgia). We examined the frequency of MDR-TB and XDR-TB, as well as the relationships among resistant strains, frequency of Beijing genotype strains, and clustering in the region.

2. Methods

2.1. Study setting

The survey was conducted in 2003–2005 in the Guliripchi TB hospital of Abkhazia, which is the only TB diagnostic centre in the region. Abkhazia is an autonomous region of western Georgia. Since 1994, this region has been in chronic conflict with Georgia and has an approximate population estimated between 150 and 190,000 inhabitants.[10] The TB case notification rate was 125/100,000 population in 2001, and 14% of the overall TB population were infected with MDR-TB strains.[11] Médecins Sans Frontières (MSF) has supported the treatment of TB patients in Abkhazia since 1999, based on World Health Organization (WHO)/International Union Against Tuberculosis and Lung Disease (IUATLD) guidelines. Since 2001, MDR- and polydrug-resistant (resistance to more than one first-line anti-TB drug but non-MDR) TB patients received second-line drugs. Indeed, beginning with 2001, the policy adopted was to initiate all TB patients (new or previously treated cases) on WHO standard short-course chemotherapy. After 2–3 months, regimens were adapted based on the DST results. The WHO Green Light Committee approved this treatment program in 2004.

2.2. Study design

All consecutive pulmonary smear-positive TB patients 18 years or older presenting to the Guliripchi TB Hospital between 2003 and 2005 were enrolled in the survey. The health authorities of Abkhazia and the Ministry of Health of Georgia approved the study. Written informed consent was obtained from included patients. TB case definitions followed WHO/IUATLD guidelines.[12] Demographic data, anti-TB treatment history, and prisoner history were recorded from patients' files.

2.3. Cultures and drug susceptibility testing (DST)

Two sputum samples were collected before initiation of anti-TB treatment and during treatment follow-up. Sputa were shipped to the Istituto Superiore di Sanità, Rome, Italy, and processed by the *N*-acetyl-L-cysteine-NaOH (NALC) method using a commercial kit (MycoPrep, Becton Dickinson, Cockeysville, MD). The sediment was suspended in PBS and inoculated into Lowenstein–Jensen (LJ) medium (100 μl) (Biomérieux, Marcy l'Etoile, France) and BACTEC MGIT 960 (MGIT) tubes (Becton Dickinson) (500 μl), according to the manufacturer's instructions. LJ slants were incubated at 37 °C in 5% CO_2 and examined weekly for 8 weeks. MTB strains were identified in positive cultures by DNA probes (Gene Probe, San Diego, Ca).[11,13] DST was carried out for first-line (isoniazid, rifampicin, streptomycin, ethambutol) and second-line (ofloxacin, kanamycin, capreomycin, ethionamide, *p*-aminosalicylic acid, cycloserine) anti-TB drugs. DST for first-line anti-TB drugs was performed using the MGIT system; DST for second-line drugs was performed using the proportion methods on 7H10 as previously described.[11] Isolated strains were stored at −80 °C in Brain Heart Infusion broth containing 20% glycerol and shipped to the Research Centre Borstel, Germany, for molecular typing. Drug-resistance case definitions followed WHO case definitions.[14] Extensively drug-resistant (XDR)-TB was defined by resistance to isoniazid and rifampicin plus resistance to any fluoroquinolone and at least one of three injectable second-line drugs (amikacin, kanamycin, capreomycin).

2.4. Molecular typing

Extraction of genomic DNA from mycobacterial strains and DNA fingerprinting, using IS*6110* as a probe, were performed according to a standardized protocol.[15] Additionally, all isolates were analysed by the spoligotyping technique.[16] Molecular typing data were analysed with Bionumerics software (version 4.5; Applied Maths, Sint-Martens-Latem, Belgium). Spoligotyping data were used to additionally confirm strain relationships and for genotype classification according to SpolDB4[17] and the MIRU-VNTRplus webpage.[18] Patients with mixed patterns indicating a double infection with two MTB strains were excluded from further analysis since no clear IS*6110* band definition was possible in mixed-strain isolates. For the cluster analysis, a cluster was defined as a minimum of two baseline (follow-up strains excluded) strains harbouring identical genotype pattern from different patients belonging to the study population.

2.5. Statistical analysis

Clinical and laboratory data were entered into a database using a SQLServer (Microsoft Visual Studio.NET 7·0) and analysed in StataSE™, 9th version, College Station, TX. Only patients' clinical and laboratory data at the time of TB diagnosis, before starting treatment, were evaluated. Results were presented for the whole study population and for groups of patients with different drug susceptibility patterns. Chi square (χ^2) test was used for comparison of proportion. Median (range) and mean (SD: standard deviation) were calculated, and T independent samples-test was used for comparison of means. P values <0.05 were considered significant. Logistic regression analysis was performed to identify patients' (age, gender, TB treatment history and prisoner history) and MTB strains' (genotype and belonging to a cluster) variables independently associated with MDR-TB. Similar analysis was performed to identify association between patients' variables, MTB genotype and clustering and between patients' variables and MTB genotype. For such analysis, the quantitative variable age was split into four subgroups (<30, 30–39, 40–49 and ≥ 50 years). Because there were very few missing observations, they were excluded from the analysis.

3. Results

Out of 405 consecutive notified smear-positive TB patients between March 2003 and September 2005, a total of 366 (90.4%) cases were screened. Of these, 326 were included in the study (Figure 1). Mean age was 41 years old (SD 14.1), 77.3% (252/326) of patients were male and 3.4% (11/326) prisoners. Twelve percent (38/324) were ex-prisoners and 39.3% (127/323) were previously treated cases (PTC).

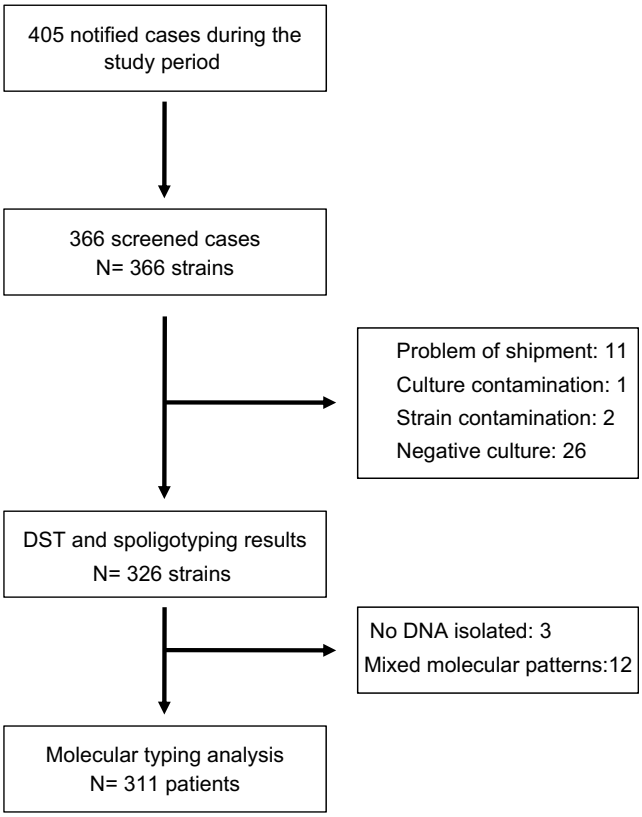

Figure 1. Study profile.

Table 1
First-line anti-TB drug resistance in 326 MTB strains.

	New cases		Previously treated cases		Total	
	No.	%	No.	%	No.	%
Total tested	196		127		326*	
Fully sensitive	90	45.9	40	31.5	131	40.6
Any resistance	106	54.1	87	68.5	195	60.4
Any H resistance	59	30.1	74	58.3	135	41.8
Any R resistance	17	8.7	51	40.2	69	21.4
Any E resistance	36	18.4	50	39.4	88	27.2
Any S resistance	75	38.3	67	52.8	144	44.6
Monoresistance						
H only	18	9.2	9	7.1	27	8.4
R only	0	0	1	0.8	1	0.3
E only	12	6.1	7	5.5	19	5.9
S only	25	12.8	4	3.1	29	9.0
H and R resistance						
MDR	17	8.7	49	38.6	68	21.0
H + R only	1	0.5	2	1.6	3	0.9
H + R + S only	5	2.6	12	9.5	17	5.3
H + R + E + S only	11	5.6	35	27.6	48	14.9
Other patterns						
H + E only	0	0	1	0.8	1	0.3
H + S only	21	10.7	8	6.3	29	9.0
H + E + S only	3	1.5	7	5.5	10	3.1
R + S only	0	0	1	0.8	1	0.3
E + S only	10	5.1	0	0	10	3.1
Number of resistant drugs						
1 drug	55	28.1	21	16.5	76	23.3
2 drugs	32	16.3	12	9.5	44	13.5
3 drugs	8	4.1	19	15.0	27	8.3
4 drugs	11	5.6	35	27.6	48	14.7

S: streptomycin; H: isoniazid; R: rifampicin; E: ethambutol.
* 3 Cases without patient's type information.

4. Drug-resistance

Data on resistance of the 326 strains to first-line drugs are presented in Table 1. Drug-resistance was more frequent among PTC, 68.5% (87/127) than among new cases (NC), 54.1% (106/196), $P = 0.01$. Overall, MDR strains were isolated from 68 patients (21%): 8.7% from NC and 38.6% from PTC.

Data on resistance to second-line drugs among MDR-TB patients are presented in Table 2. Overall, 39 (57.3%) MDR-TB patients had isolates that were also resistant to one or more second-line drugs. PTC isolates were more often resistant to second-line drugs (33/49, 67.5%) than NC isolates (6/17, 35.3%), $P = 0.02$. The frequency of XDR-TB among MDR-TB patients was 4.4% (3/68); all 3 XDR-TB patients were PTC.

5. Molecular typing

In general, a high degree of diversity of IS*6110* DNA fingerprint and spoligotyping patterns was observed among the 323 strains analysed (for three strains, DNA isolation was not successful). For 311 isolates (96.3%), single molecular typing patterns were obtained, while 12 strains (3.8%) showed mixed patterns demonstrating a double infection with two MTB strains (data not shown). Since no clear IS*6110* band definition is possible in mixed-strain isolates, the patients with mixed infections were excluded from further investigations.

Among the 311 strains included in the fingerprint analysis, 78 (25.1%) showed the typical Beijing genotype spoligotype and IS*6110* RFLP patterns. (Figure 2). However, two clearly different groups of strains could be identified within the Beijing branch: one showing RFLP patterns identical or similar to the W strain family (group 1 in Figure 2), and the other with clearly distinct RFLP patterns which have been previously described for strains from other parts of the former Soviet Union (group 2 in Figure 2).[19] Furthermore, a third group of strains had a spoligotype typical for Beijing genotype but an IS*6110* RFLP pattern less similar to the other Beijing strains (group 3 in Figure 2).

In addition, our analysis revealed the presence of a second group of closely related isolates of a previously unknown genotype, which was named ABK-1. Comparison with previous strain classifications, based on spoligotyping (SpolDB4, MIRU-VNTRplus webpage), revealed that the majority of strains had a spoligotype pattern characteristic of the Latin American Mediterranean (LAM) lineage of the TB complex.[17] Overall, 64/311 strains (20.6%) were ABK-1 strains.

General characteristics of Beijing and ABK-1 strains are presented in Table 3. Males ($P = 0.05$) and PTC ($P < 0.001$) were both

Table 2
Second-line anti-TB drug resistance in 68 MDR MTB strains, according to patient type.

	New cases $n = 17$*		Previously treated cases $n = 49$*		Total $N = 68$	
	No.	%	No.	%	No.	%
No resistance to second-line drugs	11	64.7	16	32.6	29	42.6
Any resistance to second-line drugs	6	35.3	33	67.3	39	57.3
Ethionamide	5	29.4	20	40.8	25	36.8
Kanamycin	3	17.6	18	36.7	21	30.9
Capreomycin	0	0	3	6.1	3	4.4
Ofloxacin	0	0	3	6.1	3	4.4
Resistance to >3 second-line drugs	0	0	2	4.1	2	2.9
XDR strains	0	0	3	6.1	3	4.4

* 2 cases without patient-type information.

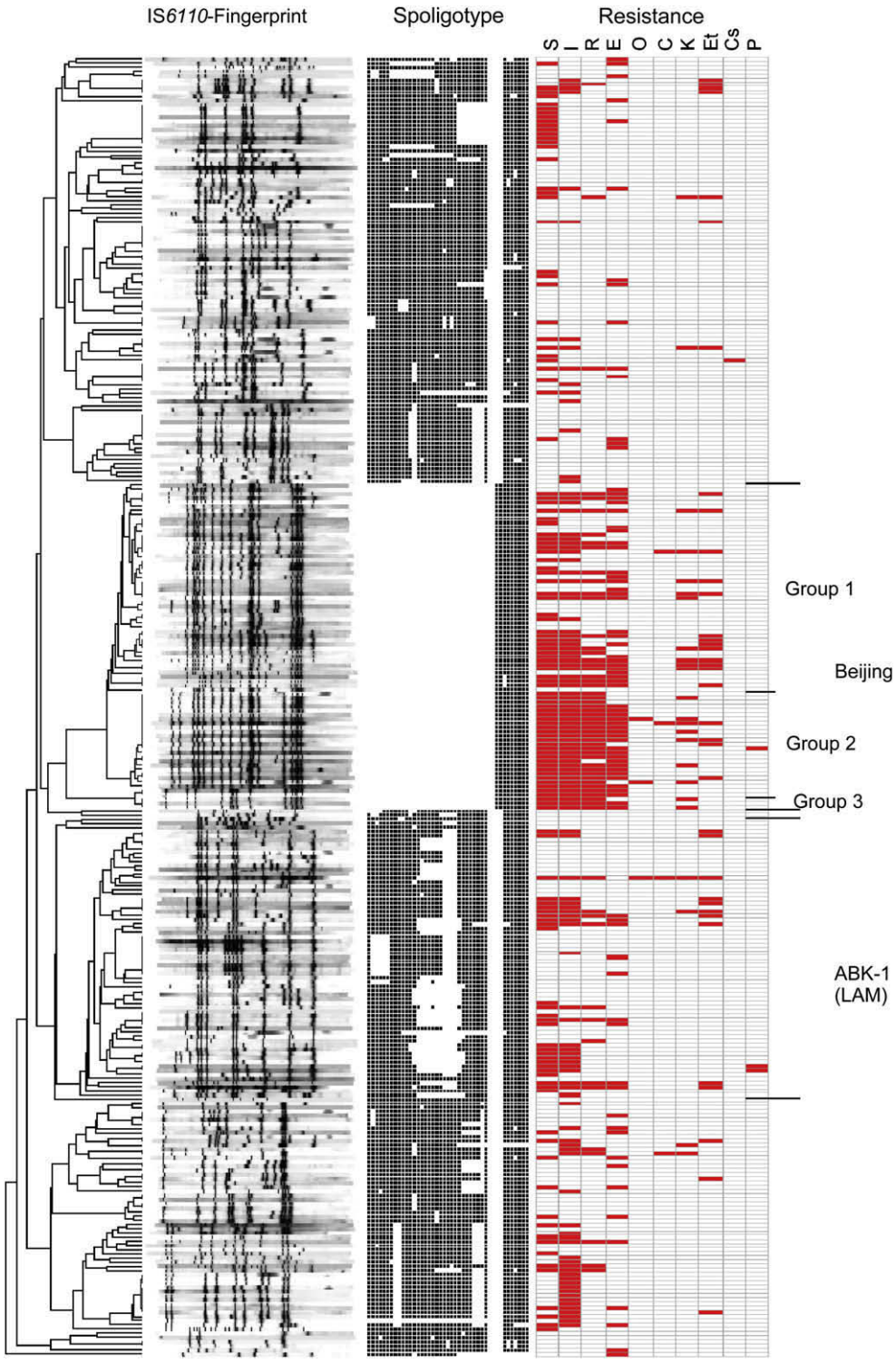

Figure 2. IS*6110* DNA fingerprint patterns, spoligotype patterns, and drug-resistance profiles of the 311 strains analysed. Banding patterns are ordered by similarity in a dendrogram as outlined in the materials and methods section. Resistance is displayed by a gray box. Abbreviations: S, streptomycin; I, isoniazid; R, rifampicin; E, ethambutol; O, ofloxacin; C, capreomycin; K, kanamycin; Et, ethionamide; Cs, cycloserine; P, para-aminosalicyclic acid.

at elevated risk of being infected with a Beijing strain. The proportion of drug-resistance is presented according to MTB genotypes in Table 4. An elevated frequency of drug resistance was found among Beijing genotypes when compared with ABK-1 genotype (Table 4).

6. Cluster analysis

Similarity analysis of strains genotyping patterns identified 165/311 patients (53.2%) with clustered baseline strains grouped in 44 clusters ranging in size from 2–14 strains (median of 3 strains per

Table 3
Patients' characteristics associated with infection by Beijing and ABK-1 strains, $N = 311$.*

Characteristics	Univariate analysis				Multivariate analysis		
	%	OR	95% CI	*P* value	OR	95% CI	*P* value
Beijing strains ($n = 78$)							
Gender							
Male	28.3	2.4	1.2–5.1	0.01	2.2	1.0–4.8	0.05
Female	14.1	1					
Age (years)							
≤30	28.6	1					
30–39	21.2	0.7	0.3–1.5	0.33			
40–49	34.8	1.3	0.6–2.8	0.43			
≥50	16.3	0.5	0.2–1.1	0.08			
Ex-prisoner							
Yes	16.7	0.6	0.2–1.4	0.22			
No	26.4	1					
Patient type							
Previously treated case	37.5	3.0	1.7–5.0	<0.001	3.0	1.8–5.2	<0.001
New case	16.5	1					
ABK-1 strains ($n = 64$)							
Gender							
Male	21.2	1.2	0.6–2.4	0.59			
Female	18.1	1					
Age (years)							
≤30	30.4	1					
30–39	26.2	0.8	0.4–1.7	0.6			
40–49	17.9	0.5	0.2–1.1	0.09			
≥50	11.6	0.3	0.1–0.7	0.01	NS		
Ex-prisoner							
Yes	27.8	1.6	0.7–3.5	0.24			
No	19.4	1					
Patient type							
Previously treated case	19.2	0.9	0.5–1.6	0.65			
New case	21.3	1					

NS: not significant.
* A total of 311 patients were included in this analysis, after exclusion of 12 strains with double infection: 78 patients infected with a Beijing strain compared to 233 infected with a strain of another genotype; 64 patients infected with a strain of ABK-1 genotype compared to 247 patients infected with a strain of another genotype. Only covariates presented in the table were included in the multivariable analysis.

cluster). The Beijing genotype (OR 2.7; 95% CI 1.4–4.8) was significantly associated with clustering after multivariate analysis, which included the age, gender, the prisoner history, the TB treatment history and the MTB genotype as covariates. In general, a positive correlation exists between the drug-resistance profiles among strains in one cluster, suggesting a significant rate of recent transmission of resistant and MDR strains in the study region (Figure 3). It should also be noted that the largest cluster (cluster 5), with 14 strains, is formed by a Beijing MDR strain. The fact that all cluster 5 strains are at least MDR, support the notion that this strain is spreading already being MDR and then potentially develops further resistances. Indeed, seven cluster 5 strains showed resistance to at least on second-line drug (Figure 3).

7. Patients' and strains' characteristics associated with MDR-TB

The results of the MDR-TB risk factors analysis are presented in Table 5. No significant difference was observed for mean age between MDR-TB (40.4, SD 12.9) and non-MDR-TB patients (43.0, SD 14.7; $P = 0.17$). The strong association between MDR-TB and clustering was not maintained after adjustment with Beijing infection in multivariate analysis.

The most striking association found, however, was the enhanced risk of patients with Beijing infection having MDR-TB (OR 25.9; 95% CI 10.2–66.0). In new cases, 10 (32.3%) of 31 Beijing strains were MDR compared with 5 (3.2%) of 157 non-Beijing strains ($P < 0.001$).

Table 4
Proportions of resistance to 1st line antituberculosis drugs according to the MTB genotype.

	$N = 311$	Beijing strain		ABK-1 strain	
		n	%	*n*	%
Resistance to					
- 0 drug	129	12	9.3	32	24.8
- 1 drug	73	10	13.7	10	13.7
- 2 drugs	42	5	11.9	13	30.9
- 3 drugs	23	14	60.9	4	17.4
- 4 drugs	44	37	84.1	5	11.4
P value*		<0.001		0.07	

* Trend Pearson chi square.

For PTC, the difference was 34/45 (75.6%) compared with 10/75 (13.3%), respectively ($P < 0.001$).

Infection with strains of the ABK-1 genotype (OR 4.0; 95% CI 1.3–12.5) and history of previous anti-TB treatment were also associated with MDR-TB (OR 6.0; 95% CI 2.7–12.9).

8. Discussion

Few population-based studies are available describing the molecular determinants of the TB epidemic, including clinical and genotypic strain characteristics, in countries of the former Soviet Union. None are in Georgia or neighbouring Caucasian countries. In this study, a systematic investigation of clinical, epidemiological, and bacteriological characteristics of more than 90% of all smear-positive pulmonary TB cases could be carried out over a 3-year time period in Abkhazia.

We report a high rate of drug resistance and specifically multi-drug resistance in both newly infected and previously treated cases, which is in agreement with observations reported from other former Soviet Union countries.[1,2,5,8] Even more worrying is the fact that, despite 100% coverage with standard short-course chemotherapy (DOTS) since 1999 and full access to free individualized drug-resistance treatments (including second-line drugs) since 2001, no reduction in MDR-TB was seen when compared with MDR rates from previous investigations in the same region (4% MDR prevalence in Abkhazia during the period 2000–2002).[1,11] This may be related to our findings, which confirm a significant association between Beijing genotype infection and MDR-TB as well as a strong expansion of one particular Beijing MDR-TB clone.

Persistence of prevalent, circulating strains of MDR-TB are likely linked to several conditions. First, the collapse of the Soviet Union, followed by the war and a long chronic conflict, has likely contributed to the current MDR situation in Abkhazia. Second, a number of factors directly related to MDR, such as the longer treatment duration, the lower cure rates, and higher default rates, compared with non-MDR-TB patients may result in greater infectiousness.[20,21] The confirmed existence of resistance to second-line drugs in Abkhazia may also result in difficulties to treat MDR-TB, further extending the period of infectivity.[11] All these factors are likely to favour the spread of MDR-TB in this region.

In Abkhazia, there was no access to rapid DST or molecular tests during the study period. Therefore, MDR-TB patients started their adapted treatment with 2–3 months delay, waiting for DST results. Such delays in receiving effective treatment may also contribute to the transmission of drug resistance and is likely to increase the risk of nosocomial transmission in settings where patients are hospitalised during the intensive phase of treatment. Furthermore, polydrug-resistant patients (10% in our study) who receive standardized first-line regimens until reading of DST results are at risk of drug-resistance amplification.[20]

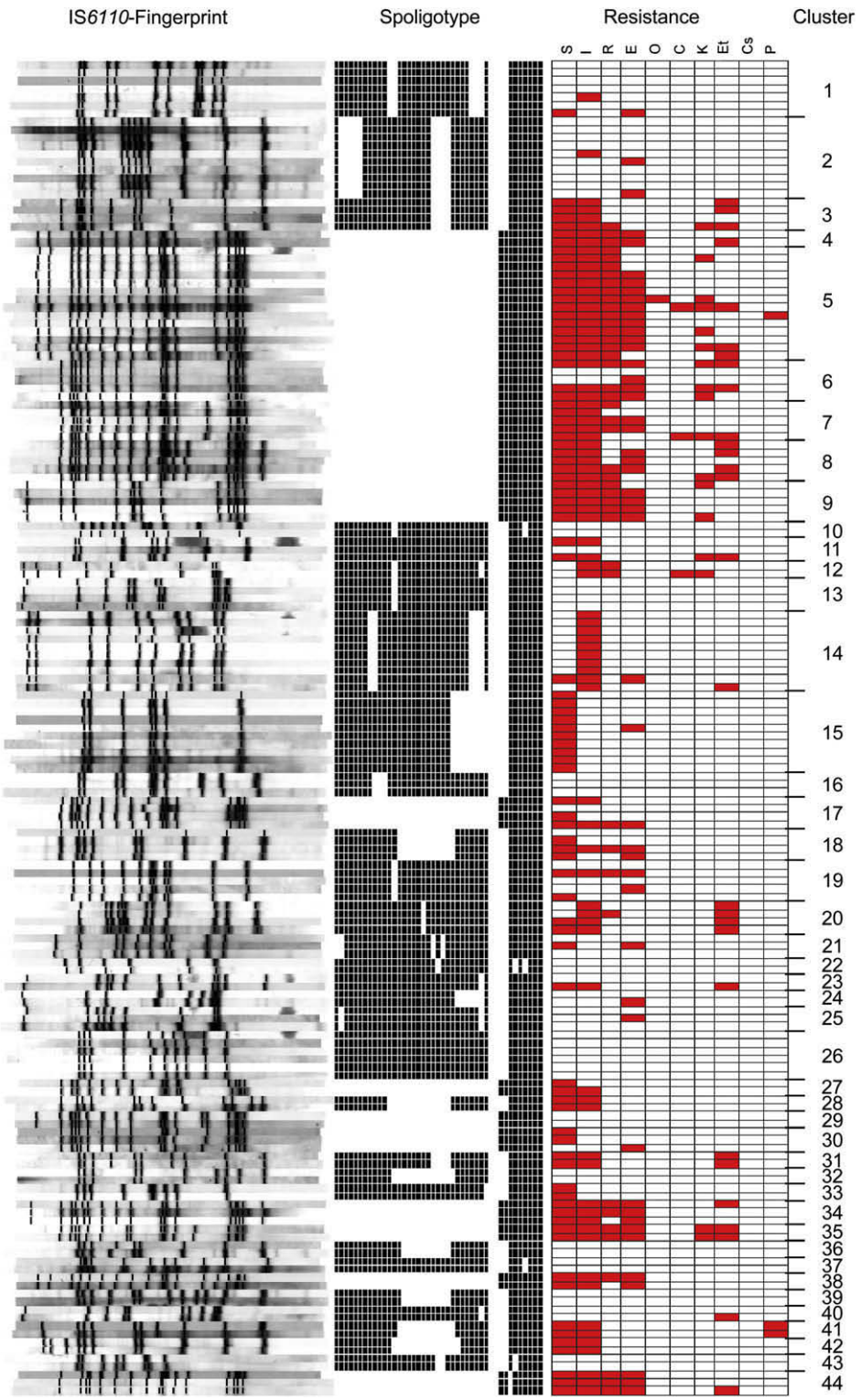

Figure 3. IS*6110* DNA fingerprint patterns, spoligotype patterns, and drug-resistance profiles of the 165 strains in clusters. Resistance is displayed by a gray box. Abbreviations: S, streptomycin; I, isoniazid; R, rifampicin; E, ethambutol; O, ofloxacin; C, capreomycin; K, kanamycin; Et, ethionamide; Cs, cycloserine; P, para-aminosalicyclic acid.

A further factor contributing to the high MDR-TB prevalence seen in Abkhazia might be the prevalence (25%) of patients infected by Beijing genotype strains. As reported in other former Soviet Union countries, Beijing genotype was also strongly associated with MDR-TB in Abkhazia.[3,8,22] A possibly higher capacity of Beijing genotype to develop further drug resistance compared with strains of other genotypes might result in a selective advantage for Beijing genotype strains in regions with high levels of drug resistance. Beijing genotype may therefore be one of the major contributors to the development and spread of TB drug resistance here and in other Eastern European countries.

Table 5
Patients' and strains' characteristics associated with MDR-TB.

$N = 311$ Characteristics	%	Univariate analysis			Multivariate analysis		
		OR	95% CI	*P* value	OR	95% CI	*P* value
Gender							
Male	21.8	1.9	0.9–4.1	0.09			
Female	12.7	1					
Age (years)							
≤30	22.0	1					
30–39	31.8	1.6	0.8–3.5	0.2			
40–49	22.0	1.0	0.4–2.2	1.0			
≥50	7.9	0.3	0.1–0.8	0.02	NS		
Ex-prisoner							
Yes	19.4	1.0	0.4–2.3	0.96			
No	19.8	1					
Patient type							
Previously treated case	36.7	6.7	3.5–12.7	<0.001	6.0	2.7–12.9	<0.001
New case	8.0	1					
Beijing strain							
Yes	59.0	20.9	10.5–41.7	<0.001	25.9	10.2–66.066.0	<0.001
No	6.4	1					
ABK-1 strain*							
Yes	12.5*	3.3	1.1–9.5	0.02	4.0	1.3–12.5	0.02
No	4.1						
Strain belonging to a cluster							
Yes	26.7	2.9	1.6–5.5	0.001	NS		
No	11.0						

A total of 311 patients were included in this analysis after exclusion of patients with mixed or double infection or absence of RFLP results. 61 MDR-TB patients were compared to 250 non-MDR-TB patients.
NS: not significant.
* Patients with Beijing strains excluded from univariate analysis.

Beijing genotype was also associated with clustering. This association was also described in Uzbekistan and Singapore.[8,9] Noteworthy, the spread of some Beijing strains in correlation with MDR was exceptional, as shown by strain cluster 5, which was responsible for 14 MDR-TB cases in the study period. The number of cases appeared to increase over time as well (data not shown), demonstrating the risk posed by such strains.

Nevertheless, the prevalence of Beijing genotype is lower in comparison with that reported for other former Soviet Union countries, with values as high as 50% in Karakalpakstan region (Uzbekistan), 66.6% in Samara region (Russia), and 70.4% in Kazakhstan.[3,8,22] This difference might be explained by the identification in 20% of all strains of a new genotype ABK-1, with characteristics typical of the LAM lineage of MTB. This genotype was also independently associated with the risk of MDR-TB. Few data are available on the epidemiological and molecular characteristics of the LAM family in former Soviet Union countries and are limited only to Russia.[4,23] In accordance with these reports, our results confirm that the LAM genotype is prevalent in a former Soviet Union country, and might contribute to the emergence of MDR-TB. This possible association is further supported by the fact that the highly transmissible XDR-TB strain from an ongoing epidemic in the KwaZulu-Natal region in South Africa also belonged to the LAM lineage of MTB.[24]

These data indicate that in areas with existing high levels of TB drug resistance and presence of strains of particular genotypes, current diagnostic and treatment strategies may actually favour the selection of highly transmissible MDR, and even XDR, clones, resulting in large TB outbreaks and ongoing transmission.[24] As in Abkhazia, these specific MTB strains have the potential to jeopardize TB control efforts in affected areas, and worldwide, if a longer time scale is considered.

Our risk factor analysis for MDR-TB was limited to the few patient characteristics collected in this observational study. We regret the absence of patients' residence addresses, which did not allow further investigation of local resistance transmission in the region. Furthermore, we could not identify the proportion of patients coming from neighbouring countries, who might have been admitted into the program during the study period. Indeed, at the time, Abkhazia was the only place with access to MDR-TB treatment free of charge in this region of the Caucasus and likely attracted patients from neighbouring countries, who failed first-line therapies. This explanation could account for the increase in MDR-TB over time among PTC. Nevertheless, identification of such patients remains difficult because patients may give a false or relative's address in order to be admitted into the program, which technically was limited to patients living in Abkhazia.

In conclusion, our study results show the difficulty to control MDR-TB in a region with pre-existing high prevalence despite the availability of treatment for drug-resistance tuberculosis in the region. This can be partially attributed to the high rate of Beijing genotype strains, and its strong association with both MDR and transmission, which are likely to be the major contributor to the spread of TB drug resistance. Thus, new approaches are urgently needed to more rapidly detect MDR-TB followed by selection of patients and adapted treatment in order to interrupt the ongoing transmission of such strains in the community in former Soviet Union countries.

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