

Determination of risk factors for the
development of tuberculosis with drug-resistant
strains of *Mycobacterium tuberculosis*
in the Arkhangelsk oblast, Russia.

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Thesis submitted as partial completion of the Master of Philosophy
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We see only what we look for.

We look for only what we know. (Goethe)

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ABSTRACT

TITLE: Determination of risk factors for the development of tuberculosis with drug-resistant strains of *Mycobacterium tuberculosis* in the Arkhangelsk oblast, Russia.

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The results of the present study were presented at The International Meeting Combating Infectious Diseases in the Baltic Sea and Barents Regions, Sig-tuna, Sweden, January 31-February 2 2000; The First Annual Students Conference, University of Bergen, Bergen, Norway, May 2000; Annual Conference at the Institute of Public Health, Oslo, Norway, December 2000; and The Second Annual Students Conference, University of Bergen, Bergen, Norway, May 2001.

DESCRIPTION OF THE PROJECT:

SETTING: Arkhangelsk, Russia, 1998-2000.

OBJECTIVE: To study *M. tuberculosis* resistance to anti-tuberculosis drugs in the Arkhangelsk oblast; and to reveal risk factors for the development of drug-resistant tuberculosis.

DESIGN: Strains isolated from 119 patients with pulmonary tuberculosis were studied by the BACTEC method. Medical records of the patients were reviewed, retrospectively, to identify factors associated with drug resistance.

RESULTS: Sixty-seven strains (56.3%) were resistant to at least one anti-tuberculosis drug. Thirty of the 119 strains (25.2%) were multi-drug resistant. All strains resistant to rifampicin were multi-drug resistant. Multidrug resistance was four times more common among previously treated patients than among new patients. The highest rates of drug resistance were observed for streptomycin and isoniazid. 40.4% and 66.7% of strains collected from new and previously treated patients were resistant to streptomycin, respectively. 37.1% and 73.3% of strains collected from new and previously treated patients were resistant to isoniazid, respectively. A history of previous or interrupted treatment for tuberculosis and being female were significantly associated with resistance to at least one anti-tuberculosis drug and multi-drug resistance.

CONCLUSION: Drug-resistant tuberculosis is an important problem in the Arkhangelsk oblast, Russia. The spread of drug-resistant strains of *M. tuberculosis* is attributed to several risk factors. A history of previous or interrupted treatment for tuberculosis and being female are significantly associated with resistance to at least one anti-tuberculosis drug and multidrug resistance. Employment in the health sector of Arkhangelsk was significantly associated with the development of drug resistance.

LIST OF ABBREVIATIONS

AIDS	acquired immune deficiency syndrome
ARTD	Arkhangelsk Regional Tuberculosis Dispensary, Arkhangelsk, Russia
Capr	capreomycin
95% CI	95% confidence interval
COPD	chronic obstructive pulmonary disease
Cycl	cycloserine
DNA	deoxyribonucleic acid
DOTS	Directly Observed Treatment Shortcourse
E	ethambutol
Eth	ethionamid
H	isoniazid
HIV	human immunodeficiency virus
IUATLD	International Union Against Tuberculosis and Lung Diseases
K	kanamycin
MDR	multi drug resistance
NAD	nicotinamideadenine dinucleotide
NIPH	National Institute of Public Health, Oslo, Norway
OR	odds ratio
PAS	para-aminosalicylic acid
PCR	polymerase chain reaction
Pr	prothionamide
R	rifampicin

RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
<i>rpoB</i>	β -subunit of the RNA polymerase
S	streptomycin
Th	thiacetazone
TB	tuberculosis
WHO	World Health Organisation
WT	wild type
Z	pyrazinamide

INTRODUCTION

The modern era of tuberculosis (TB) began in the mid-1980s. At that time it was realised that TB had not only stopped to decline in many developed countries but was actually increasing. It was realised that the disease was out of control across most of the poorest countries of the world. It was for this reason that in 1993 the World Health Organisation (WHO) declared TB to be a global emergency.¹

Globally, it is estimated that approximately one-third of the global population is infected with *Mycobacterium tuberculosis* and that 7 to 8 million new cases of TB occur each year. WHO has estimated that the total number of cases in the world will rise from 7.5 million in 1990 to 10.2 million in the year 2000.²⁻⁴ Despite of being a treatable and preventable disease, TB kills an estimated 2 million people during each year. If TB control will not be further strengthened nearly 1 billion people will be newly infected, 200 million people will get sick, and 35 million will die from TB between 2000 and 2020.⁵ TB is a leading infectious cause of death among people older than 5 years. Around 6% of all deaths worldwide are attributed to TB.⁶⁻⁸ It is expected that TB will remain one of the 10 leading causes of mortality and morbidity in the world.

Most of the TB cases and deaths from TB occur in developing countries. Increases in TB incidence have been observed in developed countries as well. In recent years, TB has become confined to definable population groups, such as immigrants from countries with a high prevalence of TB, elderly people, substance abusers, homeless, persons in correctional facilities and nursing homes. A high incidence of the disease is not unexpected in

these groups. Stress and low body weight are more common among homeless, substance abusers and elderly people and they have been reported to increase the risk of TB.

Factors associated with the resurgence of TB in many countries include the human immunodeficiency virus (HIV) epidemic, immigration from endemic areas, urban homelessness, drug abuse and insufficient attention to national TB control programme.²

The resurgence of TB has been accompanied by rising drug resistance. The spread of *M. tuberculosis* drug-resistant strains is one of the most actual problems in infectious diseases. World community is anxious about the possibility of the development of dangerous multi-drug resistant TB, that is resistance to at least rifampicin (R) and isoniazid (H).

Drug-resistant TB is alarming for several reasons. Infection with drug-resistant *M. tuberculosis* can give rise to practically untreatable forms of the disease. There are only a few effective drugs available for the treatment. Treatment of a patient with drug-resistant especially multi-drug resistant TB should include second line drugs that are less effective, have more side effects and are more expensive. Treatment of drug-resistant TB must be individualised and based on the patient's medication history and results of susceptibility test.

The multi-drug resistant TB can cause many deaths. Patients infected with resistant especially multi-drug resistant strains are less likely to be cured, especially if they are co-infected with HIV or malnourished.

The resurgence of TB and wide spread of drug-resistant TB in Russia was attributed to decreased life standard of the population, socio-economical changes in the country and unsatisfactory national TB control programme.⁹

CHAPTER I

LITERATURE REVIEW

Global TB epidemiology, reasons for TB increase

Most of TB cases and deaths in the world occur in poor countries. Increase of TB incidence has been observed in developed countries as well. In industrialised countries, more than 80% of individuals infected with *M. tuberculosis* are over the age of 50. By contrast, in the developing world, over 75% of TB cases are found in individuals below the age of 50, the most economically productive age group.¹⁰

The global increase of TB incidence has been observed since the mid-1980s. There are four principal reasons for this.

Demographic factors have played a major role in the global re-emergence of TB. Childhood mortality rates have declined much more rapidly than birth rates over the past 30 years, resulting in dramatic increase in the size of adolescents and young adult population in the world. The populations mostly of poor countries have increased. The highest incidence of TB across the world are in central Africa and Southern Asia, particularly in India, where the population increase is known to be the most rapid. Current annual population growth in these countries is about 100 million, which means that global TB incidence in absolute numbers will continue to increase by around 100.000 cases every year.²

Factors associated with the resurgence of TB in many countries include HIV epidemic. It is known that co-infection with HIV increases the risk of TB infection developing into disease by a 100 fold. Infection with HIV leads to ex-

tensive destruction of the immune defence mechanisms of the body. As a result, those infected with HIV become ill with severe and often deadly diseases to which persons without HIV infection would not usually be susceptible. In countries with high TB prevalence, HIV infection is the most important factor making a person liable to get clinical TB. Among people already infected with TB their life time risk of clinical TB is about 50% if they have been infected with HIV. This compares with a 5-10% risk if they are HIV negative. In addition HIV infection elevates the risk of rapid progression from primary infection to the disease. HIV infection has led to increase of TB incidence especially in young and middle-aged adults and it has also caused sharp increase in TB case fatality rates particularly in the absence of effective case management.^{2,11,12}

Social and economic trends have also contributed to the spread of TB. Over the past 10 years the number of less developed countries has doubled while gross national product in some middle-income countries has decreased. This has meant a decrease in the availability of resources for TB control programmes. Population movement in the form of migration of labour, general migration, armed conflicts and refugee movement is more common to take place nowadays than at any other time in history. It facilitates the increase of TB transmission. In recent years, TB has become confined to definable population groups, such as disadvantaged populations, immigrants from countries with a high prevalence of TB, refugees, displaced, the elderly, homeless, substance abusers, persons in correctional facilities and nursing homes. A high incidence of the disease in these groups is not unexpected because the rates

of TB are higher in lower socio-economic groups. Poverty leads to bad and overcrowded housing or poor work conditions. These may lower defences as well as making infection more likely. People living in these conditions are often badly nourished, suffer from alcohol abuse or drug addiction. The whole complex of poverty makes it easier for the *M. tuberculosis* to cause the disease.^{2,11,13}

During the past 4 decades national TB control programmes have failed to reduce TB transmission. Health policies in most low and middle-income countries have not given priority to TB control. Inadequately funded programmes have led to an increase in the pool of chronic infectious sources.¹¹ The overwhelming problem with the treatment of TB is that cure takes months of treatment. The great majority of TB patients in the world have poor health care facilities. Therefore patients do not complete their treatment. Premature stop of the treatment for TB results in relapse and the emergence of drug resistance.

TB epidemiology in Russia

During the 19th century the TB epidemic in Western Europe was called “The Great White Plague” because it killed up to 25% of the European population. At that time TB was practically unknown in Russia. The first cases were not reported until 1865, except those travelling to Western Europe. After a steadily increase, the TB epidemic in Russia reached its peak during the World War I (1914-1917), when about 2 million people, or about 2% of the population, died from TB.¹⁴ Subsequently, the prevalence of TB dramatically

declined in Russia, as well as in other parts of the former Soviet Union. Russian experts attributed this decline to the government sponsored TB control programme introduced in the 1920s.¹⁵

The state government actively supported measures for TB prevention and treatment. The first system to combat TB – a dispensary system for TB prevention and treatment – was established in 1918. All anti-tuberculosis organisations and institutions were nationalised in 1922. Eventually a centralised TB control system was established.¹⁶

The analysis of the main epidemiological rates of TB in Russia from 1965 has allowed to reveal different tendencies in the dynamics. The incidence rate in 1965 was 119.0/100.000 (excluding the prison system). It was decreasing until 1991. After that time a tendency of increasing incidence and mortality rate of TB has been observed. The incidence rate has increased from 34.0/100.000 (excluding the prison system) in 1991 to 85.2/100.000 (including the prison system) in 1999.¹⁶ The same tendency was observed for the mortality rate. It increased from 8.1/100.000 in 1991 to 16.5/100.000 in 1999.

In the Arkhangelsk oblast, the incidence of TB in 1965 was 119.0/100.000 (excluding the prison system), and it was decreasing until 1991. After that time the incidence rate increased from 20.7/100.000 in 1991 to 104.0/100.000 (including the prison system) in 2000. The mortality rate has also increased from 3.6 to 16.5/100.000 during the same time period.

Rates nearly hundred times higher have been recorded in prisons. The epidemiological implications of TB in prisons in the republics of the former So-

viet Union may be more serious than commonly assumed.¹⁷ Data from Marinsk (Siberia), Russia, indicated that a third of patients with TB had been in prison. A similar trend was observed in the Ivanovo oblast, Russia. About 100.000 people confined within the Russian prison system have been diagnosed active TB. About 40.000 of them had multidrug resistance (MDR). Every year the prison system of Russia releases 30.000 people into the community with active TB – about 12.000 of them with multi-drug resistant TB.¹⁸

Prisoners in countries of the former Soviet Union including Russia, like those in other parts of the world, are at increased risk for TB for several reasons. Prisoners usually come from underprivileged backgrounds and are therefore more likely to be infected with *M. tuberculosis* or even developed TB before entering prison. Poor hygiene, malnutrition, inadequate ventilation and overcrowding inside prisons provide the ideal conditions for TB spread. Prisoners and former prisoners may have an important role in TB transmission, particularly of multi-drug resistant forms in the community.^{17,19,20}

The increase of TB incidence in Russia and former Soviet Union is not associated with the HIV epidemic and demographic factors. It is a matter of fact that increase of TB rates observed during the past 10 years in Russia was not associated with the HIV epidemic. The increase of TB in Russia is rather due to poverty, malnutrition and social dislocation.²⁰

Disintegration of the Soviet Union occurred in 1991. As a result 15 sovereign states or countries and Russian Federation among them appeared. The beginning of the next 1992 year was marked by great political and economical changes. Great changes in Russia have began in socio-political sys-

tem, society structure, agency of power and administration, consciousness of Russian citizens and economy of the country. These changes resulted in deep social and economical crises. The crises led to decreased general reactivity of the population and therefore created conditions for wide spread of infectious diseases and TB among them.²¹

The economical crises happened in Russia in August 1998 has played an important role in the increase of TB epidemiological rates for the past 2 years and has created additional risk for the development of TB epidemic. The full-scale financial crisis, which burst out in August, had a clearly expressed political nature. It was a result of an inability of all the Russian governments between 1995-1998 to provide a balanced state budget through democratic procedures for mobilising a consensus of interests. In August 1998, there was a sharp jump of consumer price growth. The actual devaluation of the ruble after August 17 led to a proportional growth of prices for foods. The population in the majority of the Russian cities activated purchases of these goods expecting a further devaluation of the ruble and shortage of imported goods. The crises have impoverished population resulting in malnutrition and deteriorating living conditions.²⁰

During the past 15 years national TB control programme in Russia was not given priority because the tendency of decreasing of TB epidemiological rates has been observed since the beginning of 1970s. As a result, it led to insufficient financial support for medical structures for TB diagnosis and treatment. Financial problems resulted in the unavailability of quality drugs and irregular drug supply. The budget cuts in medical service led to inade-

quate treatment of the disease. Inadequate supply of drugs and the subsequent inability to offer a full course of treatment has led to the development of drug-resistant and multi-drug resistant TB, doubling in civilian communities from 3% to 6%, while the rate in prisons was 40%.^{22,23}

Drug-resistant TB

The resistance of certain *M. tuberculosis* strains to anti-tuberculosis drugs is not a new phenomenon. It was noted when streptomycin (S) was first used as monotherapy for TB in the 1940s. The development of multi-drug treatment regimens in the 1950s offered a way to overcome the problem. From the 1950s through the 1980s the frequency of the transmission of drug-resistant organisms was thought to be low. It was not until the early 1990s when outbreaks of multi-drug resistant TB were reported in patients with HIV infection in the United States and Europe, and the problem received international attention.^{7,8,13,24-30}

The spread of *M. tuberculosis* drug-resistant strains is one of the most acute problems in infectious diseases. Drug-resistant TB is alarming for several reasons. First, there are only a few effective drugs available. Infection with drug-resistant strain can give rise to potentially untreatable form of the disease. Second, although only about 5% of immunocompetent population infected with *M. tuberculosis* succumb to the disease, nevertheless, the disease is highly contagious.^{19,31}

Drug resistance is divided into two types: primary and secondary (or acquired) resistance.

Primary resistance is defined as resistance in persons who have never received anti-tuberculosis drugs for more than 1 month.³² These patients are initially infected with drug-resistant strains.

Acquired resistance is defined as resistance to anti-tuberculosis drugs, which arises during treatment due to poor compliance or improper management.^{4,32}

Adult patients can be infected with primary drug-resistant strain or acquire resistance to anti-tuberculosis drugs during the treatment. Usually, children have primary resistance, as they get infected from adult source with drug-resistant TB.^{33,34}

The terms *acquired drug resistance* and *primary drug resistance* suggest that the exact causative nature of drug resistance is known. Patients may not disclose prior treatment for TB due to several reasons. If this occurs, the term *primary drug resistance* may be used inappropriately, as resistance may have been acquired during previous concealed treatment. On the other hand, patients who fail treatment may do so because their strain was initially resistant to anti-tuberculosis drugs and not because it acquired resistance during the treatment. That is why the WHO and International Union Against Tuberculosis and Lung Diseases (IUATLD) recommend to use terms *drug resistance among new cases* and *drug resistance among previously treated cases*.²²

Drug resistance among new cases (formerly: *primary drug resistance*) is the presence of drug-resistant strain of *M. tuberculosis* in a newly diagnosed patient who has never received anti-tuberculosis drugs or has received them for less than 1 month.²²

Drug resistance among previously treated cases (formerly: *acquired drug resistance*) is that found in a patient who has previously received at least 1 month therapy with anti-tuberculosis drugs.²²

The real magnitude of *M. tuberculosis* drug resistance world-wide is not known.³⁵ In 1995, overall primary resistance rates in the world were described to be 0-16.9% for H, 0-3.0% for R, 0-4.2% for ethambutol (E) and 0.1-23.5% for S.¹² Rates of acquired resistance were higher. The spread of multi-drug resistant TB was relatively low in most countries where representative studies have been conducted. Primary MDR was described to be 0-10.8% and acquired MDR was found to be 0-48.0%.³⁵

The problem of drug resistance existed in Russia even during the Soviet era (in the mid-1980s). Rates of drug resistance have significantly increased during the past decade. According to survey from the North-Western part of Russia, primary drug resistance to at least one anti-tuberculosis drug increased from 17.0% to 24.0% during 1991-1994. Acquired drug resistance existed on a remarkable scale in the North-Western Russia even 10 years ago, but since that time resistance patterns have gradually shifted towards MDR.^{36,37}

The latest data on TB drug resistance in Russia are described by the WHO/IUATLD surveillance and presented in the Table 1.²²

Table 1 Indicators of TB drug resistance in the Ivanovo and Tomsk oblasts, Russia, presented in the WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance, 2000²²

Oblast	New cases						Previously treated cases					
	Resistance to (%)				MDR (%)	Any resistance (%)	Resistance to (%)				MDR (%)	Any resistance (%)
	H	R	E	S			H	R	E	S		
Tomsk	19.4	7.9	7.0	24.9	6.5	29.0	42.7	31.0	21.1	53.4	26.7	57.8
Ivanovo	22.1	15.8	9.9	18.0	9.0	32.4	33.3	42.6	29.6	46.3	25.9	68.5

MDR = multidrug resistance; Any resistance = resistance to at least one drug; H = isoniazid; R = rifampicin; E = ethambutol;

S = streptomycin

In the Arkhangelsk oblast, primary drug resistance was 15% in 1991, while acquired drug resistance was 60%. In 2000 these parameters of drug resistance increased to 33% and 85%, respectively (unpublished data from the Arkhangelsk Regional Tuberculosis Dispensary (ARTD)).

MDR

MDR is defined as in vitro resistance of *M. tuberculosis* strains to two or more anti-tuberculosis drugs. MDR state in mycobacteriology refers to simultaneous resistance to at least R and H (with or without resistance to other drugs). Multi-drug resistant TB causes most concern because mortality is significantly higher among persons infected with multi-drug resistant strain than of those infected with sensitive strain. Second or third line drugs are necessary for treatment and they have greater side effects. Patients with multi-drug resistant TB remain infectious for longer time that increases the risk of infection transmission.^{35,38,39}

The emergence of multi-drug resistant TB overlapped with the resurgence of TB. Nosocomial outbreaks of multi-drug resistant TB have been reported in the USA, France and other countries.^{13,27,40} These outbreaks occurred in hospitals, nursing homes, shelters for the homeless, residential facilities for patients with HIV infection and acquired immune deficiency syndrome (AIDS) and correctional institutions.⁴¹

Clinical manifestation of multi-drug resistant TB has many features that have been described among patients with HIV infection and TB. The manifestation is characterised by fever, cough, dyspnea and night sweats.⁴⁰

Outcomes of multi-drug resistant TB is usually poor with high mortality rate. Persons with multi-drug resistant TB were reported to have more likely alveolar infiltrates, cavity pulmonary lesions, reticular interstitial infiltrates, and respiratory insufficiency than those infected with susceptible strain.⁴²

Multi-drug resistant TB should be suspected in the following cases:

- Patients in hospitals or prisons known to be experiencing outbreaks of multi-drug resistant TB;
- Patients from geographic areas where multi-drug resistant TB is common;
- Patients known to be at high risk for multi-drug resistant TB, those infected with HIV and having AIDS, intravenous drug users or homeless;
- Patients who have relapsed after prior treatment.⁴³

Molecular mechanisms of drug resistance development

M. tuberculosis is naturally resistant to many antibiotics particularly those belonging to the β -lactam, macrolide or tetracycline families. This is a result of its highly lipophilic cell envelope acting as an efficient barrier.^{19,31}

According to the recent advances of molecular biological technics, some of the genetic mechanisms of drug resistance has been uncovered. The mechanisms are chromosomal, caused by one or more mutations in independent genes. Drug-resistant organisms are produced by random mutations occurred spontaneously at different gene loci at a low but predictable frequency in wild type (WT) strains even before the strains come in contact with anti-tuberculosis drugs. These gene mutations occur at fairly consistent rates – approximately 10^{-6} for H and S, 10^{-8} for R and 10^{-4} for E.

When two or more drugs are considered together the value becomes the product of the individual probabilities. The mutation rate for resistance to more than one drug is calculated by multiplying the rates for the individual drugs. For example, the mutation rate for resistance to both H and R is approximately 2.56×10^{-10} . The probability of MDR development is dependent on the number of mutant bacilli.^{34,44,45}

Wild mutations of *M. tuberculosis* are considered to be equally distributed in all parts of the world. A high primary resistance rate in a certain region results from an inefficient national TB control programme in the past, because of a huge transmission of drug-resistant strains in that region during the past years.^{38,46}

Mutations can produce bacilli resistant to any of the anti-tuberculosis drugs. The probability for resistance is very high for less effective anti-tuberculosis drugs such as thiacetazone (Th), ethionamide (Eth), capreomycin (Capr), cycloserine (Cycl) and viomycin; intermediate for drugs such as H, S, E, kanamycin (K) and para-aminosalicylic acid (PAS); and the lowest for R. Consequently the probability of a mutation is directly proportional to the bacterial load. Resistance to a drug does not confer any selective advantage to the bacterium unless it is exposed to that drug.^{38,47}

An untreated TB cavity has 10^7 - 10^{10} organisms, thousands of which are resistant to a single drug due to a random mutation. The chance of having an organism with WT resistance to two drugs is the product of individual probabilities. Drug-resistant TB occurs when drug-resistant bacilli outgrow drug susceptible bacilli. Drug-resistant mutants are selected when therapy is

inadequate and when a single drug is used to treat a large population of bacilli. If a cavitary lesion is treated with a single effective drug the susceptible bacilli are eliminated but the small number of mutants resistant to the drug continue to multiply. After 2 weeks to several months of treatment with the single drug, the susceptible bacilli will be eliminated but the resistant bacilli will survive and continue to multiply causing clinical drug resistance to the particular drug. If the single anti-tuberculosis therapy is replaced by another effective drug than the second drug will kill bacilli sensitive for the second drug but the small number of mutants resistant to the second drug will survive. As a result mutants resistant to two drugs are selected. The patient may eventually have bacilli resistant to two or more drugs. The patient will relapse with the disease that is completely resistant to that drugs.⁴⁸

Selection favoring the resistant mutants occurs during several cycles of killing (when drugs are taken) and regrowth (when drug taking stops). During each cycle the size of bacilli population regrows back with the subsequent increased proportion of resistant bacilli. Four mechanisms favoring resistant mutant selection will be described bellow. The first two occur during phases of bacterial inhibition or killing, and the second two occur during subsequent regrowth.⁴⁸

First mechanism is known as bacterial effects during initial killing. Any regimen containing H kills *M. tuberculosis* more rapidly than those with R (but without H) do at the start of treatment. This suggest that at the commencement of an H/R regimen, H-resistant mutants are selected because they can only be killed by R, whereas sensitive bacilli can be killed by the more bacteri-

cidal H. There would only be a short period for selection since early bactericidal activities are only different during the first two days of treatment, after which the rate of kill is similar in all regimens. The bactericidal population at the start of treatment is high and likely to contain mutants resistant to a single drug. This mechanism would produce resistance if poor compliance took the form of cycles in which the patient took the combined preparation for 2-3 days, followed by at least several days when no drugs were taken, to allow the bacterial population to return to the pre-treatment state of growth. Although relevant early bactericidal activity data are incomplete; there would probably be a slower selection of mutants resistant to a drug with a high early bactericidal activity, other than H, in any drug combination.⁴⁸

The second mechanism is called monotherapy during sterilisation of special populations. A hypothesis to explain the high sterilising activities of R and pyrazinamide (Z) has assumed that there are special populations of semi-dormant organisms selectively killed by R (because they show spurts of metabolism) and by Z (when the bacilli are in a very acid environment). Resistant mutants should then survive better than sensitive organisms. If we consider a single uninterrupted phase of sterilisation, the chances of resistant mutants surviving will depend on their prevalence within the original special bacterial population in the lesions of the patient.⁴⁸

The third effect is sub-inhibitory drug concentrations during regrowth. Anti-tuberculosis drugs may be present at sub-inhibitory concentrations during periods of regrowth. It can not inhibit growth completely, but it slows the regrowth down. The slowing of growth applies to sensitive bacilli, but not to

those resistant to drug *A*. Mutants resistant to *A* are selected. Second drug *B* does not affect this selection since, this happens during a period of regrowth. Thus *B* can not prevent some regrowth and have an equal effect on sensitive organisms and mutants resistant to *A*. There must also be a similar selection of resistance to *B* if sub-inhibitory concentrations of *B* are present. This mechanism would be most effective for drugs that have a high therapeutic margin, particularly H, for a long half-life, because their sub-inhibitory concentrations would exist after those for other drugs.⁴⁸

The fourth mechanism is bacteriopausal effects during regrowth. If we consider the situation when treatment is prescribed only with H and R. After the first few doses the sensitive bacilli are fully loaded with H. If there is then a gap in drug taking, the sensitive bacilli remain inhibited for several days, since 5-7 days is the maximal lag period following exposure to H. The lag period after exposure to R is much shorter. As a result of these differences in lag periods, H-resistant mutants would be selectively encouraged at the time of regrowth; they would not be inhibited by R because of its short lag period. This mechanism is likely to be most active in selecting mutants resistant to H and S since these two drugs have the longest lag period.⁴⁸

It is difficult to know which of these four mechanisms is most effective in producing resistant bacilli because of their complexity. The second mechanism (monotherapy during sterilisation) would only produce resistance at relapse and select for monoresistance to R. The other three mechanisms would mainly select for resistance to H initially, but could go on to cause resistance to other drugs being given. All mechanisms would operate irrespective of the

number of drugs being taken, provided that there were repeated cycles of killing and regrowth.⁴⁸

Treatment by single drug and changes of therapy regimens create possibility for development of resistance for several drugs. In a large population of resistant mutants, additional mutations can occur resulting in doubly resistant mutants. Patients acquire resistance to several drugs through repetition of the mutation process. Serial selection of drug resistance is the predominant mechanism for the development of MDR. The patients with MDR constitute a pool of chronic infections, which propagate primary MDR. Delayed recognition of drug resistance is one of the major factors contributing to multi-drug resistant TB development. These results in prolonged exposure to drugs that are virtually ineffective, creates possibility for resistant microorganism to multiply and prolonged infectiousness.^{41,48}

Similar situation occurs when the regimen contains multiple drugs but only one drug to which the infecting bacilli are susceptible. This can happen when primary drug resistance is not suspected or when a single drug is added to a failing regimen. These regimens are equivalent to single drug therapy, and they can select multi-drug resistant organisms. Bacilli may also be exposed to a single drug for long periods of time when medications are not taken as prescribed, that allows drug-resistant organisms to emerge.

When the treatment regimen contains two effective bactericidal drugs each drug eliminates the subpopulation of organisms with WT resistance to the other drug. But the possibility for resistant bacilli to survive exists. Effective cure can be provided by regimens containing at least two drugs to which *M.*

tuberculosis strain is sensitive. This statement was proved by controlled trials. They demonstrated that combined regimens were more efficient than single drug regimens in treating TB and preventing the emergence of drug resistance.^{34,49}

Anti-tuberculosis drugs have individual targets in *M. tuberculosis* responsible for the resistance development. R has a single target in 69 bp polymorphic fragment of the gene encoding for the β -subunit of the ribonucleic acid (RNA) polymerase (*rpoB*) responsible for its resistance. Two possible mechanisms of R resistance are mutations of RNA polymerase and alterations in cell wall permeability that inhibit drug uptake.⁴² The RNA synthesis is inhibited in bacteria by R bonding to the deoxyribonucleic acid (DNA) dependent RNA polymerase.

Other anti-tuberculosis drugs have more complicated mechanisms as they may have several targets responsible for drug resistance. Mutations in *katG* and *inhA* genes cause resistance to H. *KatG* gene encodes the haem-containing enzyme, catalase-peroxidase. There are biochemical and genetic proofs that H undergoes a peroxidative reaction catalysed by catalase-peroxidase, in which it is transformed into an exquisitely potent bactericidal derivative. The precise nature of this compound remains obscure. It has been proposed that H may be converted into isonicotinic acid, an analogue of nicotinic acid the precursor for nicotinamideadenine dinucleotide (NAD) synthesis. It remains unclear whether catalase-peroxidase directly transports H into the cell or acts indirectly by maintaining the membrane transport system. Muta-

tions in *katG* may affect enzyme levels and thus may be responsible for varying degrees of resistance to H.⁴²

InhA gene encodes an enzyme involved in mycolic acid production. Most isolates that contain *inhA* mutations in the absence of mutations in *katG* have relatively low level H resistance, less than or equal to 1 µg/ml, while isolates with a complete deletion of *katG* can have minimum inhibitory concentrations as high as 50 µg/ml.^{42,50} However, there may be another target confined to *M. tuberculosis*.

The potential mechanisms of resistance to H include:

- mutations that inhibit the suppression of the mycolitic acid synthesis pathway;
- mutations in the biosynthesis pathway that would inhibit the assembly of the catalase-peroxidase of *M. tuberculosis* to an active compound;
- inactivation of an inhibitor of NAD glycohydrolase, which depletes intracellular concentrations of NAD.

Molecular basis for S resistance had been extensively studied on several other eubacteria where it was known to result from mutations in genes for ribosomal subunits. S interferes with protein synthesis in mycobacteria by binding to the 16S ribosomal RNA causing misreading of the genetic code and inhibition of translation. Mutations in the 16S rRNA gene and gene *strA* – encoding for ribosomal protein s12 which stabilises a functionally important pseudoknot structure formed by 16S rRNA – confer resistance to S in *M. tuberculosis*.^{43,50}

Initial reports described the E mechanism as binding to the cell wall. Later inhibition of arabinogalactan synthesis that is the component of the cell wall was presented at the most relevant function. Genetic explanation of E resistance is mutation in *embCAB* gene cluster.⁵⁰ More recently the inhibition of glucose conversion into the precursors used for the synthesis of cell wall polysaccharides such as arabinogalactan, arabinomannan and peptidoglycan has been proposed. A non-contiguous genomic region has been cloned and presented in a preliminary report as containing determinants, which encode the putative target of E.

Risk factors for the development of drug resistance

A limited number of studies has been performed to estimate the problem of drug resistance and determine risk factors for its development in Russia. We would like to distinguish and discuss separate risk factors for the development of acquired or resistance among previously treated cases and primary or drug resistance among new cases.

Risk factors for the development of resistance among previously treated cases

Previous treatment for TB, non-compliance to treatment or failure to complete a curative course of therapy and inadequate management by a physician during the treatment for TB are considered to be risk factors for the development of acquired drug resistance or resistance among previously treated cases.

Previous treatment for TB is considered by many authors to be either important cause or risk factor for the development of drug resistance.^{25,34,38,42,45} The success of the drug treatment of TB has been the catalyst for the emergence of drug resistance. Patients have been allowed to take their medications at home completely unsupervised. There is a danger that if the patient is sent home with three separate drugs, he or she might take a single drug at a time. In the patient with extensive lung disease taking a single drug just a few days may allow drug resistance to emerge. If a patient happens to be resistant to one drug and takes a combination of two drugs including the one to which he is resistant, drug resistance to the second drug will emerge. Similarly if the patient is resistant to two drugs, and takes these two drugs and a third only then resistance to the third will emerge and so on.⁵¹

Patients with drug resistance often have a history of not taking the prescribed anti-tuberculosis medications for at least 1 month or have not received the recommended retreatment regimen. This allows bacilli to accumulate mutations and acquire resistance.²⁵

Non-compliance due to patient's related reasons is the most important factor in the emergence of acquired resistance. The reasons for non-compliance considered as patient's responsibility usually are multifaceted resulting from characteristics of the individual patients and quality of their social and economic background. Some potential barriers to successful treatment include the need for long term (6 to 12 months) and complicated drug regimens, the cost in time and money, long waits in crowded public health facilities, contradictory expectations and beliefs between patients and

health care providers, communication difficulties and transportation. Homelessness, psychiatric diseases, alcoholism, drug addiction and substance abuse can predict non-compliance. Persons who use alcohol and other drugs may be at risk for repeated exposure to those who have TB.^{34,38,45,52}

Poor compliance can also be caused by health system failure. Failure to complete a curative course of therapy can be due to improper prescribed treatment that is wrong choice of anti-tuberculosis drugs or prescription therapy not according to the standard treatment for different case categories.^{25,34}

Inadequate therapy can be due to suboptimal dosages and reduced absorption of the drugs.⁴³ Several reports suggest that malabsorption of anti-tuberculosis drugs may favour the development of drug resistance. Alterations of pharmacokinetics can lead to subtherapeutic concentrations of anti-tuberculosis and thereby promote the emergence of drug resistance within individual patients.³⁸

Many cases of drug-resistant TB arise from inadequate management by a physician or ineffective TB control programme. The most common errors are addition of a single drug to a failing regimen, inappropriate single drug therapy, an inadequate initial regimen, failure to recognise primary or acquired resistance and failure to recognise or deal with non-adherence to prescribed treatment.³⁴

Risk factors for the development of resistance among new cases

Contact with a person having infectious, drug-resistant TB, HIV infection and immunocompromised conditions other than HIV infection are the risk factors for the development of primary or resistance among new cases.

Contact with a person, who has infectious, drug-resistant TB is a significant factor for primary resistance. Recent nosocomial outbreaks demonstrate a strong correlation between previous exposure to a patient who has infectious, multi-drug resistant TB and the subsequent development in the contact of multi-drug resistant TB. Several factors are important and they should be taken into consideration in risk assessment. They are the infectiousness of the possible source; the closeness and intensity of the exposure and the contact's likelihood of exposure to persons with drug susceptible TB. Any person who shares the air space with a patient with multi-drug resistant TB for a relatively prolonged time (e.g., household member, hospital roommate) is at higher risk for infection than those with a brief exposure to a multi-drug resistant TB patient, such as a one-time hospital visitor. Exposure of any length in a small, enclosed, poorly ventilated area is more likely to result in transmission than exposure in a large, well-ventilated space. Exposure during cough inducing procedures (e.g., bronchoscopy, endotracheal intubation, sputum induction, and administration of aerosol therapy) may greatly enhance TB transmission is also more likely to result in infection.¹²

HIV infection is another risk factor for the development of primary drug resistance.^{42,43,53} One of the most alarming consequences of dual infection

with HIV and *M. tuberculosis* has been the emergence of MDR. A transmission of multi-drug resistant TB among individuals in contact tended to occur very quickly. These included patients, prison guards and health care workers. Persons with HIV infection are more likely to be infected with TB if exposed. A type of catastrophic spread of these multi-drug resistant TB infections in nosocomial or closely similar congregational settings might thus occur. The HIV epidemic may have a significant effect on the spread of primary drug resistance in communities with co-existent HIV and drug-resistant TB since contacts of HIV infected people with *M. tuberculosis* are more likely to result in active disease and more quickly compared with contacts of infected immunocompetent people. This explains the high level of primary drug-resistant TB when combination of TB, inadequate treatment and HIV infection is observed.^{25,31,38}

The diagnosis of TB in HIV infected persons is sometimes delayed because of the unusual radiographic presentations of TB, co-infection with other pulmonary pathogens to which patients' symptoms were attributed, and the overgrowth of *M. tuberculosis* in the laboratory by other mycobacteria. Delays in diagnosis lead to delays in the initiation of isolation and treatment and prolonged infectiousness. Prolonged infectiousness promotes further transmission.

A high risk for developing multi-drug resistant TB includes immunocompromised conditions other than HIV infection. They include conditions requiring prolonged high dose corticosteroid therapy and other immunosuppressive therapy including bone marrow and organ transplantation, chronic renal

failure, some haematological disorders e.g., leukemia and lymphoma, systemic diseases of connective tissues, and acquired immunodeficiency condition other than HIV infection.

Restriction fragment length polymorphism (RFLP) analysis in mycobacteriology

In the past few years there has been a large increase in the application of molecular techniques to various aspects of mycobacteriology. The discovery of insertion elements in mycobacterial species has made it possible to differentiate strains for either epidemiological studies or to determine sources of mycobacterial contamination that could lead to false diagnosis of the disease. Chromosomal DNA is first digested, electrophoretically separated and then transferred to a membrane for hybridisation with labelled genetic probes designed to detect copies of the insertion element. The tracking of patient to patient transmission of *M. tuberculosis* is of highest concern. Standardised methods for the detection of copies of insertion element IS6110 have been internationally accepted for typing *M. tuberculosis*.⁵⁴

RFLP has proved to be accurate in the determination of *M. tuberculosis* strain relatedness and in the recognition of distinct outbreaks. Systematic DNA fingerprinting of *M. tuberculosis* isolates proved to be a powerful tool for the study of interstrain relations in community. RFLP analysis with the insertion sequence IS6110 is useful for the characterisation of *M. tuberculosis* strains, the identification of outbreaks and for tracing of nosocomial infections.⁵⁵⁻⁵⁷

Comparison of resistant and susceptible *M. tuberculosis* strains using RFLP analysis has been performed in several studies. Some researchers tried to identify whether certain risk factors for TB were associated with specific RFLP types. For example, injection drug users, alcoholism, pulmonary infection as the major site of the disease and homelessness were significantly associated with having a shared DNA fingerprint. Significant association was not found between DNA fingerprint clustering and race, sex, HIV status or non-intravenous drug use.⁵⁸

The Arkhangelsk study

The Arkhangelsk oblast is situated in the North of the European part of Russia and covers a territory of 589.900 sq. km. The administrative organisation of the oblast is composed of 20 districts (raions), 13 towns, 38 settlements of urban type, about 4.000 rural settlements. The population of the oblast is 1.493.000 inhabitants (according to the estimation made by the end of 1998), with a density of 2.5 inhabitants per sq. km. The urban population is 1.086.500 and the rural population is 373.400 inhabitants. The major part of the population lives along railroads and in the basins of big rivers – Severnaya Dvina, Vaga, Pinega, Onega, and Mezen. In the economic system of Russia the Arkhangelsk oblast stands out as an area of timber, woodworking and pulp and paper industries, timber export and fish industry.

Phthisiopulmonology service of the Arkhangelsk oblast was founded in 1923. It was presented by hospitals and departments for TB treatment situated in every raion of the oblast. After the Second World War service was

centralised. ARTD started to play the key role in TB control. The hospital in Majmaksa (Arkhangelsk) was opened in 1962. It provided treatment mainly for chronic patients. Small TB hospitals in the oblast were closed at the end of 1960s. An inpatient department in the ARTD was opened in 1986.

Nowadays TB service of the oblast is presented by the ARTD, Majmaksa hospital, 50 beds ward in Kotlas, 25 beds ward in Velsk, dispensary in Severodvinsk, sanatorium in Shenkursk and TB department in Regional Psychiatric Hospital.

The ARTD receives TB patients from the whole oblast and the city. The ARTD is divided in outpatient and inpatient departments. Inpatient department occupies 3 floors, each with one ward with 60 beds in each ward. The capacity is 180 patients. Some efforts are made to separate patients with multi-drug resistant TB. The outpatient department is divided in 2 parts. One that looks after the different 13 districts in the municipality of Arkhangelsk city. Ten doctors work there. One doctor in principle takes care of one district. The second part of outpatient department takes care of all raions in the oblast. Three oblast doctors work there. The oblast doctors receive patients' medical documents concerning patients (x-ray, bacteriological examination, medical record etc) for consultation. Oblast doctor has the responsibility for several raions.

If any person living in Arkhangelsk city or oblast is considered by the primary health service to suffer from TB he is then referred to the district TB doctor in the ARTD. If examination supports the TB diagnosis, the patient is usually admitted to inpatient department of the ARTD for the intensive phase of treatment. The continuation phase of treatment can be given either in

inpatient or outpatient departments of the ARTD for patients from the city of Arkhangelsk. Patients treated at inpatient department stay during the whole course in the ARTD. Patients receiving treatment in continuation phase at outpatient department come to the ARTD to get drugs. These patients come daily with the exception of Saturdays and Sundays. Some patients (20%) get drugs for 10 days at a time (continuation phase of category I with EH), and some (20%) are day-patients staying for the whole day and getting food in addition to their drugs. Others stay at home and are taken care by the nurse in the district, but the district TB specialist will also make home visits. The continuation phase of treatment for patients from the oblast can be given either in inpatient or outpatient department of the ARTD or in certain districts where the staff has been trained in the new TB strategy. Some patients can be referred to Majmaks hospital for continuation phase.

History of TB treatment in Arkhangelsk

S and PAS were introduced in phthisiopulmonology practice in Arkhangelsk in 1940s, H, Cycl and Th – in 1960s and R – in 1973. The treatment including H, S and PAS has been used since the end of 1960s. Doctors started to prescribe combination of H, R and S after introduction of R as anti-tuberculosis agent. The treatment for TB usually included 3 drugs. Severely ill patients were given 4 drugs: H, R, S and E. Treatment was prescribed for 12 months. After 3 months of treatment S was substituted by E. Treatment took place at inpatient department of the ARTD. After 6-8 months of treatment patients were discharged and continued treatment at outpatient department.

At this stage they received 2 anti-tuberculosis drugs: H and E or H and prothionamide (Pr). Individual treatment was started to be prescribed after introduction of R and E into practice. Prophylactic courses of chemotherapy with either H or other 2 anti-tuberculosis drugs were administered during 2 or 3 months until 1998.

In 1998, the implementation of treatment based on the Directly Observed Treatment Shortcourse (DOTS) strategy was started. Nowadays treatment for TB is prescribed according to standard treatment regimens for different case categories (Table 2).⁵⁹ Anti-tuberculosis drugs and dosages are presented in the Table 3.⁵⁹

In many cases the intensive phase of treatment is prolonged until results of susceptibility test are known. The results of sensitivity test are usually available after 10-12 weeks. Decisions are then made concerning further treatment according to the methodology recommendations.⁵⁹

Table 2 Chemotherapy regimens for different case categories prescribed in the Arkhangelsk oblast⁵⁹

Category	TB patients	Alternative treatment regimens	
		Intensive phase	Continuation phase
I	new case	2EHRZ	4HR; 6HE; 4H ₃ R ₃
II	relapse, failure, default	2KEHRZ/1EHRZ	5HRE; 5H ₃ R ₃ E ₃
III	new smear neg PTB, (primary TB in children, extra-PTB without severe complications)	2HRZ	4HR; 6HE; 4H ₃ R ₃
IV	chronic case, MDR	individual treatment regimens according to results of susceptibility tests	

TB = tuberculosis; E = ethambutol; H = isoniazid; R = rifampicin; Z = pyrazinamide; K = kanamycin; new smear neg PTB = new smear negative case of pulmonary tuberculosis; extra-PTB = extrapulmonary tuberculosis; MDR = multidrug resistance

Table 3 Essential anti-tuberculosis drugs and dosages prescribed in the Arkhangesk oblast⁵⁹

Drug	Mode of action	Max daily dose	Recommended dose (mg/kg)		
			Daily	Intermittent	
				3x/wk	2x/wk
H	bactericidal	300 mg	5(5-10)	10(8-12)	15(13-17)
R	bactericidal	600 mg	10(8-12)	10(8-12)	10(8-12)
Z	bactericidal	2.5 g	25(20-30)	35(35-40)	50(40-60)
S	bactericidal	1.0 g	15(12-18)	15(12-18)	15(12-18)
E	bacteriostatic	1.6 g	15(15-20)	30(25-35)	45(40-50)
Eth, Pr	bactericidal	750 mg	12(10-20)	-	-
Ofl	slight bactericidal	800 mg	12(7.5-15)	-	-
K, A	bactericidal	1.0 g	15(12-18)	15(12-18)	15(12-18)
Capr	bactericidal	1.0 g	15(12-18)	15(12-18)	15(12-18)
Cycl	bacteriostatic	750 mg	10-20	-	-

3x/wk = 3 times a week; 2x/wk = 2 times a week; H = isoniazid; R = rifampicin; Z = pyrazinamide; S = streptomycin; E = ethambutol; Eth = ethionamide; Pr = prothionamide; Ofl = ofloxacin; K = kanamycin; A = amikacin; Capr = capreomycin; Cycl = cycloserine

OBJECTIVES OF THE STUDY

Superior objective – to reveal risk factors for the development of drug-resistant TB in the Arkhangelsk oblast, Russia.

Specific objectives:

1. To identify social, demographical and medical factors associated with the development of drug-resistant TB.
2. To compare the resistant and susceptible *M. tuberculosis* strains circulating in the Arkhangelsk population using RFLP analysis.

CHAPTER II

MATERIALS AND METHODS

Patients and bacterial strains

The study was performed in the Arkhangelsk oblast on patients with pulmonary TB diagnosed and treated in the ARTD.

Population at risk was 1.493.000 inhabitants of the Arkhangelsk oblast (according to estimation made by the end of 1998).

Target population consisted of TB patients from the Arkhangelsk oblast.

Study sample included patients from the Arkhangelsk oblast with new and previously treated cases of pulmonary TB. Patients diagnosed in the ARTD were selected according to the inclusion criteria.

Inclusion criteria:

1. Patients with new cases of pulmonary TB from whom strains have been collected during a 3 month period (June, July and August) in 1998.
2. Patients with new and previously treated cases of pulmonary TB from whom strains have been collected during the second half of 1999 and during the first 3 months of 2000.
3. The strains must have been collected from the patients before the prescription of treatment with anti-tuberculosis drugs.

Sample size

The sample size for the study was calculated according to the WHO and IUATLD recommendations for sample size calculation for a survey on the prevalence of anti-tuberculosis drug resistance.³² Sample size was calculated using the lowest prevalence of resistance estimated according to the results of susceptibility tests performed at the National Institute of Public Health (NIPH), Oslo, Norway, on strains collected in the Arkhangelsk oblast.⁶⁰ The lowest rate of drug resistance was observed for R. Estimated prevalence for R resistance was $p=0.09$. $a=0.05$ was chosen as allowable error. Number of observations was calculated as $N= 4p(1-p)/a^2 = 4 \times 0.09 \times (1-0.09)/0.05^2 = 131$. The sample size was increased by 15% to account for unexpected losses. The final sample size consisted of 150 *M. tuberculosis* strains.

Collected sample

A total of 146 *M. tuberculosis* strains isolated from patients with pulmonary TB in the ARTD during 1998–2000 were cultivated on Lowenstein-Jensen media and forwarded to the NIPH, Reference Laboratory for Tuberculosis, for further analysis. Thirteen strains were either heavily contaminated or died during transportation. Among the remaining 133 strains, one strain (the first one isolated) per patient was selected. Then, the final sample consisted of 119 *M. tuberculosis* strains: 43 strains from 1998; 46 strains from 1999; and 30 strains from 2000.

Representativeness of the sample

Any person living in the Arkhangelsk oblast suspected to have TB by a general practitioner were routinely referred to the ARTD. Referral criteria included suspicion of TB and did not depend on severity of the disease or possible development of drug resistance. The ARTD performed registration of patients and treatment. Selection of the sample did not exclude any patients with TB from other hospitals as registration and TB treatment was exclusively performed in the ARTD.

Private practice existed at a limited degree. There were only few private doctors in Arkhangelsk. They were employed by state hospitals and had private practice as extra time work. Private doctors did not have responsibility to diagnose and treat TB. If a private doctor suspected TB in any patient then the patient was referred to the ARTD.

According to the system described above all patients with TB in the Arkhangelsk oblast had an equal access to the ARTD and possibility to be included in the study sample. The study sample included 57 of the 119 (47.9%) patients representing the city of Arkhangelsk and surrounding areas. The rest 62 of the 119 (52.1%) patients represented all 20 raions of the oblast (Table 4).

Table 4 Patients representing the city of Arkhangelsk with surrounding areas and raions of the oblast

Area	No of patients (%)
The city of Arkhangelsk and surrounding areas	57 (47.9)
Isakogorskyi raion	3 (2.5)
Lomonosovskiy raion	16 (13.6)
Oktyabrskiy raion	9 (7.6)
Solombalskyi raion	10 (8.5)
Novodvinsk	6 (5.1)
Primorskyi raion	10 (8.5)
Tsyglomen	2 (1.7)
Non	1 (0.8)
Arkhangelsk oblast	62 (52.1)
Cholmogorskyi raion	4 (3.4)
Kargopolskyi raion	4 (3.4)
Konoshskiy raion	2 (1.7)
Kotlaskiy raion	2 (1.7)
Krasnoborskyi raion	1 (0.8)
Lenskyi raion	1 (0.8)
Leshukonskyi raion	3 (2.5)
Mezenskyi raion	4 (3.4)
Nyandomskiy raion	5 (4.2)
Onezskiy raion	8 (6.8)
Pinezskiy raion	5 (4.2)
Plesetskyi raion	8 (6.8)
Shenkurskyi raion	4 (3.4)
Ustyanskyi raion	8 (6.8)
Verchne-Toemskiy	1 (0.8)
Vinogradovskiy raion	2 (1.7)

The sample size was collected during fixed period of time as it is described in the inclusion criteria. The analysis of quarterly reports on registration for the past 2 years allowed to conclude that there was no seasonal variations of TB diagnosis in the Arkhangelsk oblast. However a sharp increase of TB patients occurred in the III quarter of 1999. This was due to the change in the registration system. Since that time patients with TB diagnosed in the prison system of the Arkhangelsk oblast have been included in the reports.

Individual analysis of data collection forms (Appendix) and records in the laboratory of the ARTD allowed to conclude that strains were collected from all new and previously treated patients during the 3 month period in 1998. The rest strains were collected during the second half of 1999 and the first 3 months of 2000 on the first grow basis. Strains were collected from culture positive cases of pulmonary TB. Collection of sputum for culture examination was performed routinely from every patient at the point of diagnosis. The strains were collected from patients with pulmonary TB who have been registered in the quarter III and IV in 1999. Patients from the prison system were not included in the sample. During that period 148 patients were notified. Summary of the quarterly reports on registration for the III and IV quarter in 1999 and characteristics of patients from whom the strains were collected are presented in the Table 5.

Table 5 Characteristics of patients with pulmonary TB registered in the III and IV quarter in 1999 in the ARTD and characteristics of patients with pulmonary TB whose strains were collected for the study

The Arkhangelsk oblast (excluding the prison system) n = 148 (%)		Strains collected in the III, IV quarter n = 76 (%)		Total sample n = 119 (%)	
new	treated	new	treated	new	treated
111(75.0)	37(25.0)	54(71.1)	22(28.9)	89(74.8)	30(25.2)

Data collection

Medical records of the 119 patients were reviewed, retrospectively, in order to identify factors associated with the development of drug resistance. Demographic factors such as age and gender; social factors including smoking habit, alcohol abuse and having been in prison; and medical factors such as presence of diabetes, gastric ulcer and chronic obstructive pulmonary disease (COPD) prior to TB diagnosis, possible contacts with another patients suffering from TB, and information about present treatment for TB were analysed.

Strain susceptibility testing

Susceptibility test to anti-tuberculosis drugs: H, R, E and S – was performed by the radiometric broth method (BACTEC, Becton Dickinson Diagnostic Systems, Towson, MD)⁶¹⁻⁶³

The BACTEC method is a modification of the conventional proportion method. Instead of an agar base the test uses Middlebrook 7H12 broth with a

radiolabeled fatty acid substrate. Growth of *M. tuberculosis* in the medium releases $^{14}\text{CO}_2$ in the bottle that is measured radiometrically. The amount of growth, indicated by changes in the growth index (ΔGI) in the medium with known drug concentrations as compared to that in the control bottle, has been correlated to a presence or absence of resistance in 1% of the inoculum. If an isolate grows beyond a specific growth index compared with the control it is considered resistant to that specific agent.⁶³⁻⁶⁵

The concentrations of the anti-tuberculosis drugs used for the test were as follows: H, 0.2 $\mu\text{g/ml}$; R, 2.0 $\mu\text{g/ml}$; E, 7.5 $\mu\text{g/ml}$; and S, 6.0 $\mu\text{g/ml}$.

In order to identify the mutations in the *rpoB* gene associated with R resistance, the Inno-Lipa Rif. TB Assay (Innogenetics N.V., Belgium) was performed for every strain of *M. tuberculosis* resistant to R by the BACTEC method, following the instructions of the manufacturer.^{36,66}

Target DNA was amplified with the reagents of the kit. The downstream primer was biotinylated at the 5' end. Polymerase chain reaction (PCR) amplification consisted of an initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, and terminal elongation at 72°C for 10 min. Amplified biotinylated DNA material was hybridised with specific oligonucleotide probes immobilised as parallel lines on membrane-based strips which was carried out by incubation in a shaking water bath at 62°C for 30 min. Streptavidin labeled with alkaline phosphatase was added to detect any biotinylated hybrid previously formed. Incubation with bromochloroindolylphosphate and nitro blue tetrazolium in dimethylformamide (BCIP/NBT) chromogen resulted in a pur-

ple/brown precipitate. The presence of *M. tuberculosis* complex in the sample was monitored by the *M. tuberculosis* complex specific probe. The reactivity of an amplified fragment with the WT probes S1, S2, S3, S4 and S5 allowed an easy detection of the mutations that lead to R resistance in *M. tuberculosis*.^{36,66}

RFLP analysis

RFLP analysis (extraction of DNA, as well as the subsequent Southern blotting, hybridisation and detection) was performed according to standardised methodology.⁶⁷⁻⁶⁹ Briefly, cells were harvested by centrifugation and lysed with lysozyme and sodium dodecyl sulfate and genomic DNA was isolated by using CTAB (cetyltrimethylammonium bromide). DNA was restricted with *PvuII* (Boehringer Mannheim, Germany) according to the manufacturer's instructions and electrophoresed in 0.8% agarose gel. After staining with ethidium bromide, the DNA fragments in the gel were photographed, and then transferred to a GeneScreen Plus membrane by the alkaline transfer procedure. The membrane was hybridised with DIG-labeled probe IS6110.^{68,69} The results of hybridisation were inspected visually, scanned and compared by Gel Compar software version 4.1 (Applied Maths, Kourkai, Belgium) by the unweighted pair – group method of arithmetic averaging with the Dice coefficient.

Statistical analysis

Epi Info version 6.04b (Centers for Disease Control and Prevention, USA; WHO, Geneva, Switzerland) was used for the main statistical analysis. Associations between categorical variables were assessed by the χ^2 test and Fisher's exact test for values less than five. Differences between groups were expressed as odds ratio (OR) with 95% confidence intervals (95% CI). Student's *t*-test was used to test for differences in means of continuous variables. A *P* value of < 0.05 was chosen as the level of significance.

Definitions

Cases were defined as patients with new and previously treated pulmonary TB whose *M. tuberculosis* strains were resistant to at least one drug.

Controls were defined as patients new and previously treated pulmonary TB whose *M. tuberculosis* strains were susceptible.

Pulmonary cases of TB included both sputum smear positive and sputum smear negative cases.⁷⁰

New cases were patients who have never previously been treated for TB for more than 1 month.⁷⁰

Previously treated cases were patients with a history of previous treatment with anti-tuberculosis drugs for at least 1 month.³²

Resistance to the first line anti-tuberculosis drugs (H, R, E, S) was defined according to the results of susceptibility test by the BACTEC method.

Any kind of resistance was defined as resistance to at least one anti-tuberculosis drug.

MDR was defined as resistance to at least H and R.³²

Drug resistance among new cases (formerly: “primary drug resistance”) was a resistance of *M. tuberculosis* strains isolated from newly diagnosed patient who has never received anti-tuberculosis drugs or has received them for less than 1 month.²²

Drug resistance among previously treated cases (formerly: “acquired drug resistance”) was a resistance of *M. tuberculosis* strains isolated from previously treated patients.²²

CHAPTER III

RESULTS

Drug resistance of the M. tuberculosis strains

A total of 119 strains collected from patients with pulmonary TB in the Arkhangelsk oblast were identified as *M. tuberculosis* and tested by the BAC-TEC method for susceptibility to the first line anti-tuberculosis drugs: E, H, R and S. The susceptibility pattern to R, H, S and E of the 119 *M. tuberculosis* strains are represented in the Table 6.

Fifty-two of the 119 (43.7%) strains were susceptible to all tested anti-tuberculosis drugs. Monoresistance to S, H and E was detected in 8 (6.7%), 4 (3.4%) and 4 (3.4%) strains, respectively. Resistance to two drugs was detected in 17 of the 119 (14.3%) strains, to three drugs in 7 of the 119 (5.9%) strains, and to four drugs in 27 of the 119 (22.7%) strains. Thirty of the 119 (25.2%) strains were multi-drug resistant. All strains resistant to R were multi-drug resistant.

Strains were collected from 89 (74.8%) newly diagnosed patients and from 30 (25.2%) previously treated patients. MDR among previously treated patients was four times higher than among new cases (Table 7). Resistance to at least one drug was found in 44 of the 89 (49.4%) strains collected from new cases and 23 of the 30 (76.7%) strains collected from previously treated cases. The highest rates of drug resistance were observed for S and H in both groups of strains: 40.4% and 66.7% of strains collected from new and previously treated patients were resistant to S, respectively; 37.1% and 73.3% of strains collected from new and previously treated patients were resistant to H,

respectively (Table 7). MDR existed at alarming rates. A total of 12 of the 89 (13.5%) strains isolated from new cases were resistant to R and H, while MDR among strains from previously treated cases was 60.0%. All multi-drug resistant strains were also resistant to S and most of them also to E.

Table 6 Susceptibility pattern of *M. tuberculosis* strains isolated from 119 patients with pulmonary TB in the Arkhangelsk oblast, Russia, 1998-2000, determined using the BACTEC method

Rif	H	S	E	No new cases n = 89 (%)	No previously treated cases n = 30 (%)	Total cases n = 119 (%)
R	R	R	R	11 (12.4)	16 (53.4)	27 (22.7)
R	R	R	S	1 (1.1)	2 (6.7)	3 (2.5)
S	R	R	R	3 (3.4)	1 (3.3)	4 (3.4)
S	R	R	S	13 (14.6)	1 (3.3)	14 (11.7)
S	R	S	S	2 (2.2)	2 (6.7)	4 (3.4)
S	R	S	R	3 (3.4)	0 (0)	3 (2.5)
S	S	R	S	8 (8.9)	0 (0)	8 (6.7)
S	S	S	R	3 (3.4)	1 (3.3)	4 (3.4)
S	S	S	S	45 (50.6)	7 (23.3)	52 (43.7)

R = resistant; S = susceptible

Table 7 Resistance of *M. tuberculosis* strains isolated from 119 patients with pulmonary TB in the Arkhangelsk oblast, Russia, 1998-2000, to the first line anti-tuberculosis drugs, determined using the BACTEC method

	New case	Previously treated case
	n = 89 (%)	n = 30 (%)
Resistance to ethambutol	20 (22.5)	18 (60.0)
Resistance to isoniazid	33 (37.1)	22 (73.3)
Resistance to rifampicin	12 (13.5)	18 (60.0)
Resistance to streptomycin	36 (40.4)	20 (66.7)
Resistance to any one drug	44 (49.4)	23 (76.7)
MDR	12 (13.5)	18 (60.0)

MDR = multidrug resistance

The 30 strains resistant to R with the BACTEC method were investigated by Inno-Lipa Rif. TB Assay to identify the mutations associated with R resistance in the Arkhangelsk oblast. Amplicons of the 69 bp polymorphic fragment of the *rpoB* gene obtained from these specimens were hybridised with Lipa to confirm resistance to R and detect the mutation in the core region of *rpoB* gene. Inno-Lipa Rif. TB Assay confirmed resistance to R of all 30 strains resistant by the BACTEC method. Seven different hybridisation patterns were represented (Table 8). Possible mutations in the *rpoB* gene of *M. tuberculosis* and corresponding Inno-Lipa patterns are represented in the Table 9.

The most common Inno-Lipa pattern was R5, that was revealed in 21 of the 30 (70.0%) strains. Other types of Inno-Lipa patterns were Δ S1, Δ S4, R2, Δ S3 and Δ S5, that were found in 10.0%, 6.7%, 3.3%, 3.3% and 3.3%, respectively.

One strain produced a certain type of binding pattern. It showed resistant R4_a pattern and at the same time it had bands typical to the WT *M. tuberculosis* strain.

Table 8 Inno–Lipa patterns of 30 *M. tuberculosis* strains resistant to R by the BACTEC method

Inno-Lipa pattern	n = 30 (%)
R5	21 (70.0)
Δ S1	3 (10.0)
Δ S4	2 (6.7)
R2	1 (3.3)
Δ S3	1 (3.3)
Δ S5	1 (3.3)
WT/R4 _a	1 (3.3)

WT = wild type

Table 9 Mutations in *rpoB* gene of *M. tuberculosis* and corresponding Inno-Lipa patterns

Mutated <i>rpoB</i> Codon	<i>rpoB</i> mutation	Amino acid change	Inno-Lipa pattern
None	none	none	WT
513	CAA → CTA	Gln → Leu	ΔS1
516	GAC → GTC	Asp → Val	R2
516	GAC → GGC	Asp → Gly	ΔS2
516	GAC → TAC	Asp → Tyr	WT/ΔS2
526	CAC → TAC	His → Tyr	R4 _a
526	CAC → GAC	His → Asp	R4 _b
526	CAC → CTC	His → Leu	ΔS4
531	TCG → TTG	Ser → Leu	R5
531	TCG → TGG	Ser → Trp	ΔS5
533	CTG → CCG	Leu → Pro	ΔS3
564	CCT → CTT	Pro → Leu	WT/R5

C = cytosine; A = adenosine; G = guanosine; T = thymine; Gln = glutamine; Leu = leucine; Asp = asparagine; Val = valine; Tyr = tyrosine; His = histidine; Ser = serine; Trp = tryptophan; Pro = proline; WT = wild type

RFLP analysis of the M. tuberculosis strains

The 119 strains were analysed by the RFLP using IS6110 as a hybridisation probe. The number of IS6110 copies varied between 7 and 17, with a mean number of 12.4 copies. Seventy RFLP patterns were revealed among 119 strains, as shown on the Figure 1. Of the 70 RFLP patterns, 59 (84.3%) were unique and 11 (15.7%) were presented by 2–14 strains with identical RFLP pattern. These strains were clustered, meaning that each of them had a hybridisation pattern identical to at least one other strain. The largest epidemiological cluster comprised 14 strains; other clusters included 11, 9, 8, 4 and 2 strains. The five largest clusters included both R-susceptible and R-resistant organisms. Within the same cluster *M. tuberculosis* strains had different susceptibility patterns and mutations in the *rpoB* gene responsible for resistance to R, as shown in the Table 10.

Table 10 Resistance pattern in relation to clusters among *M. tuberculosis* strains

Cluster size	Resistance to				No. strains	Inno-Lipa pattern (No. strains)
	E	H	Rif	S		
14	S	S	S	R	6	
	R	R	R	R	4	ΔS1 (3) R5 (1)
	S	R	S	R	2	
	R	S	S	S	1	
	S	S	S	S	1	
11	R	R	R	R	9	R5 (8) ΔS5 (1)
	R	R	S	R	1	
	S	R	S	R	1	
9	S	R	R	R	3	R5 (2) R2 (1)
	R	R	R	R	2	R5 (2)
	S	R	S	R	2	
	S	S	S	S	2	
8	R	R	R	R	6	R5 (6)
	S	R	S	R	1	
	S	S	S	S	1	
4	S	S	S	S	3	
	S	R	S	R	1	

Rif = rifampicin; E = ethambutol; H = isoniazid; S = streptomycin; R = resistant; S = susceptible

Description of epidemiological clusters

The eleven clusters are identified by the capital letters *A* through *E* in order of decreasing size. The summary and characteristics of the five largest epidemiological clusters based on the RFLP findings are represented in the Table 11.

Cluster A comprised 14 strains (4 strains from 1998, 5 strains from 1999 and 5 strains from 2000). Twelve of the 14 (85.7%) strains were collected from newly diagnosed patients with pulmonary TB. The age of the patients varied from 19 to 41 years, with a mean age of 30.0 years. Half of the patients were male. Three of the 14 (21.4%) patients have been living in the Arkhangelsk oblast for less than 10 years. Two of the 14 (14.3%) patients had been in prison. *M. tuberculosis* strains collected from 13 of the 14 (92.9%) strains were resistant to at least one drug. Four of the 14 (28.6%) strains were multi-drug resistant. Inno-Lipa test showed that Δ S1 pattern was dominant, as it was represented in 3 of the 4 R-resistant strains. R5 was the Inno-Lipa pattern of the last strain (Table 10).

Cluster B comprised 11 strains (1 strain from 1998, 6 strains from 1999 and 4 strains from 2000). Six of the 11 (54.5%) strains were collected from newly diagnosed patients. The age of the patients varied from 25 to 70 years, with a mean age of 41.1 years. Seven (63.6%) patients were male. One of the 11 (9.1%) patients has been living in the Arkhangelsk oblast for less than 10 years. Six patients lived in the city of Arkhangelsk itself and 5 patients were from elsewhere in the oblast. Two of the 11 (18.2%) patients had a history of having been in prison. All strains collected from the patients within the *cluster B* were resistant to at least one anti-tuberculosis drug. Nine of the 11 (81.8%)

strains were multi-drug resistant. R5 Inno-Lipa pattern was dominant, as it was observed in 8 of the 9 R-resistant strains. Δ S5 was the Inno-Lipa pattern (Table 10) of the remaining strain.

Cluster C consisted of 9 strains (7 strains from 1998, 1 strain from 1999 and 1 strain from 2000). Six of the 9 (66.7%) strains were collected from new patients with pulmonary TB. The age of the patients varied from 19 to 56 years, with a mean age of 37.7 years. Eight (88.9%) patients were male. One of the 9 (11.1%) patients has been living in the Arkhangelsk oblast for less than 10 years. Six patients lived in the city of Arkhangelsk and 3 patients were from elsewhere in the oblast. Four of the 9 (44.4%) patients had been in prison. *M. tuberculosis* strains collected from 7 of the 9 (77.8%) strains were resistant to at least one drug. Five of the 9 (55.6%) strains were multi-drug resistant. R5 Inno-Lipa pattern was dominant, as it was found in 4 of the 5 R-resistant strains. The other found pattern was R2 (Table 10).

Cluster D comprised 8 strains (2 strains from 1998, 3 strains from 1999 and 3 strains from 2000). Half of the strains were collected from new patients with pulmonary TB. The age of the patients varied from 21 to 72 years, with a mean age of 43.5 years. Six (75.0%) patients were male. All patients were born in the Arkhangelsk oblast. Two patients lived in the city of Arkhangelsk and 6 patients outside. One of the 8 (12.5%) patients had been in prison. *M. tuberculosis* strains collected from 7 of the 8 (87.5%) patients within the *cluster D* were resistant to at least one drug. Six of the 8 (75.0%) strains were multi-drug resistant. All R-resistant strains had R5 Inno-Lipa pattern.

Cluster E comprised 4 strains they all were from the year 1998. Three of the 4 strains (75.0%) were collected from newly diagnosed patients. The

age of the patients varied from 31 to 61 years, with a mean age of 43.3 years. All patients were male. They all have been living in life in different districts of the Arkhangelsk oblast during the whole their life. They all had an evidence of being in prison. Three of the 4 (75.0%) strains of *cluster E* were susceptible to all drugs. One strain (25.0%) was resistant to both H and S.

Cluster F comprised 3 strains, they all were from the year 1998. Two of the 3 (66.7%) strains were collected from new patients. The age of the patients varied from 27 to 46 years, with a mean age of 36.3 years. Two of the 3 patients were male. All patients have been living in the Arkhangelsk oblast during their whole life. One of the 3 (33.3%) patients had been in prison. One of the 3 (33.3%) collected strains was resistant to H, the other two strains were susceptible.

Cluster G comprised 2 strains they all were from the year 1999. The strains were collected from new patients. The age of the patients was 40 and 46 years. Both patients were male. They have been living in the Arkhangelsk oblast during their whole life. One of them had been in prison. One of the 2 strains was resistant to S, the other was susceptible.

Cluster H comprised 2 strains, both from the year 2000. One strain was collected from newly diagnosed patient, the other was collected from patient previously treated for TB. The age of the patients was 39 and 42 years. Both patients were male. They have been living in the Arkhangelsk oblast during their whole life and both had been in prison. One of the 2 strains was resistant to H, the other was susceptible.

Cluster I comprised 2 strains, both isolated in 1998, from new cases. The age of the patients was 37 and 39 years. Both patients were male, both

have been living in the Arkhangelsk oblast during their whole life in flats situated in the same house. The *M. tuberculosis* strains collected from these patients were resistant to H and S.

Cluster J comprised 2 strains (1 strain from 1999 and 1 strain from 2000), collected from new patients 39 and 47 years old with pulmonary TB. Both patients were male. One patient had been living in the Arkhangelsk oblast for less than 10 years, one patient had been in prison. Both strains collected from these patients were susceptible to all drugs.

Cluster K comprised 2 strains, isolated in 1998, from new cases. The age of the patients was 45 and 68 years. One of the patients was male. Both patients have been living in the Arkhangelsk oblast during the whole life. One patient had been in prison. One of the 2 strains was resistant to S, the other was susceptible.

Table 11 Characteristics of the five largest epidemiological clusters

	Cluster A n=14 (%)	Cluster B n=11 (%)	Cluster C n=9 (%)	Cluster D n=8 (%)	Cluster E n=4 (%)
No of IS6110	14	16	16	17	15
No of new cases	12(85.7)	6(54.5)	6(66.7)	4(50.0)	3(75.0)
Mean age	30.0	41.1	37.7	43.5	43.3
Male	7(50.0)	7(63.6)	8(88.9)	6(75.0)	4(100.0)
< 10 years	3(21.4)	1(9.1)	1(11.1)	0(0)	0(0)
Being in prison	2(14.3)	2(18.2)	4(44.4)	1(12.5)	4(100.0)
Any resistance	13(92.9)	11(100.0)	7(77.8)	7(87.5)	3(75.0)
MDR	4(28.6)	9(81.8)	5(55.6)	6(75.0)	0(0)

< 10 years = living in the Arkhangelsk oblast less than 10 years; any resistance = resistance to at least one drug; MDR = multidrug resistance

Patients

All patients presented with pulmonary TB and were HIV negative. The age of the patients varied from 16 to 80 years, with a mean age of 38.9 years. Eighty-nine of the 119 patients (74.8%) were male.

Patients' weight varied from 37.0 kg to 100.0 kg, with a mean weight of 61.3 kg. Body mass index (BMI) was calculated to estimate type of body constitution. BMI varied from 16.3 to 36.2, with a mean of 21.3. Forty-one of the 119 (34.5%) patients were underweight, that is BMI less than 20.0. Seventy-four of the 119 (62.2%) patients had normal weight, that is BMI between 20.0 and 24.9. Two of the 119 (1.7%) patients were overweight, that is BMI between 25.0 and 29.9. Two of the 119 (1.7%) patients were obese, that is BMI more than 30.0.

Almost all patients were native born in the Arkhangelsk oblast. Only 6 of the 119 (5.0%) patients have been living in the Arkhangelsk oblast for less than 10 years.

Marital status was assessed in terms of legal status. A total of 58 of the 119 (48.7%) patients were married, 49 (41.2%) were single, 7 (5.9%) were divorced and 5 (4.2%) were widowed (Figure 2).

The level of education was estimated as the highest last grade attained (Figure 3). The majority of the patients, that is 113 of the 119 (95.0%) patients, had secondary education. Three of the 119 patients (2.5%) had higher education. Two of the 3 patients with higher education were medical doctors employed in the ARTD. The third patient with higher education was an advocate employed in the prison of Arkhangelsk. Three of the 119 (2.5%) patients had only primary education, due to mental disability.

Unemployment was relatively common among patients with TB, as 56 of the 119 (47.1%) were unemployed. Forty-six of the 119 (38.7%) patients were employed, 14 patients (11.8%) were retired and 3 patients (2.5%) were students (Figure 4). Five of the 46 (10.9%) employed patients were occupied in the health sector as doctors, nurses and nurse assistants. Twenty-nine of the 46 (63.0%) employed patients had physical work and 12 (26.1%) employed patients had mental work (Figure 5).

Residence was classified as private flats, homes and hostels. Ninety of the 119 (75.6%) patients lived in flats, 22 of the 119 (18.5%) patients lived in houses, 5 of the 119 (4.2%) patients lived in hostels and 2 of the 119 (1.7%) patients were homeless. The number of rooms in private house, flat or apartment in a hostel varied from 1 to 6, with a mean number of rooms of 2.3. The number of persons living together in the same house, flat or hostel apartment varied from 1 to 7, with a mean number of persons of 3.0. The number of persons per room in a housing unit varied from 0.2 to 4.0, with a mean number of persons per room of 1.4. Crowded accommodation was determined as more than 2 persons per room in housing unit. Thirty of the 117 (25.6%) patients had crowded living conditions (2 patients were excluded, as they were homeless).

Figure 2 Marital status of 119 patients with pulmonary TB

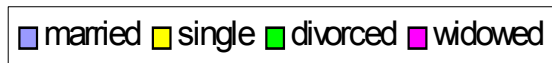
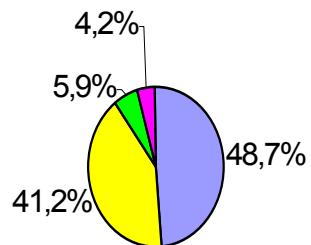


Figure 3 Education of 119 patients with pulmonary TB

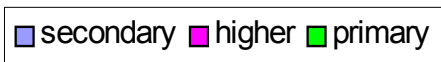
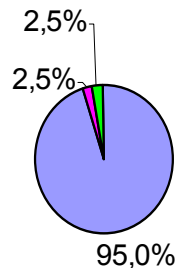


Figure 4 Occupation of 119 patients with pulmonary TB

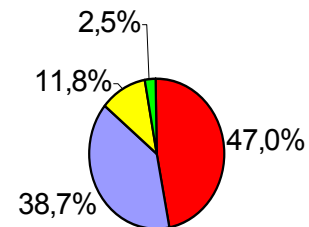
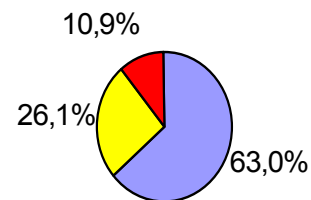


Figure 5 Occupation of 46 employed patients with pulmonary TB



As shown on the Figure 6, habit of smoking and alcohol abuse was found in 92 of the 119 (77.3%) and 54 of the 119 (45.4%) patients, respectively. None of the patients was addicted to drugs. Thirty-six of the 119 (30.3%) patients had been in prison during 0.3-17.0 years, with a mean duration of 5.5 years, prior to the disease. A total of 23 of the 36 (63.9%) former prisoners have been in prison for less than 5 years.

Contact with another patient with TB was determined as living in the same flat or house or working together. It was relevant for 43 of the 119 (36.1%) patients (Figure 7). Eighteen of the 43 (41.9%) patients who have had contact with another TB patient had contact with sick relative (parent, child, spouse, brother or sister). Two patients had a contact with sick parent employed in the health sector of the Arkhangelsk oblast. Nine of the 43 (20.9%) patients communicated with a sick neighbor. Eight of the 43 (18.6%) patients have had contact with another TB patient while they were in prison. Seven of the 43 (16.3%) patients had contact with TB patients at their place of work, as 2 of the 7 were employed in the prison of Arkhangelsk, the other 5 were medical doctors, nurses and nurse assistants. One of the 43 (2.3%) patients indicated that the contact person with TB was a former prisoner.

Some patients had an indication of having chronic disease prior to TB diagnosis. Twenty-seven of the 119 (22.7%) patients have had a history of COPD, that is bronchial asthma, pneumonia more than twice during life period or chronic bronchitis. Seven of the 119 (5.9%) patients have had diabetes and 8 of the 119 (6.7%) patients have had gastric ulcer prior to TB diagnosis.

A previous treatment for TB was defined as treatment with anti-tuberculosis drugs documented in medical records of the ARTD, as all pa-

tients in the Arkhangelsk oblast suspected to have TB are referred to the ARTD. Thirty of the 119 (25.2%) patients had an evidence of previous treatment for TB. Detailed information about their treatment is presented on Figure 8. Thirteen of the 30 (43.3%) previously treated patients had been treated for TB in prison. Two of the 30 (6.7%) previously treated patients had been treated irregularly due to drug supply problem. Six of the 30 (20.0%) previously treated patients were treated not according to the standard treatment regimens for different case categories. The diagnosis and treatment of these 6 patients took place in prison during the 1987–1997. At that time the DOTS strategy was not yet implemented in the Arkhangelsk oblast. One of these patients received mono-therapy with H during 6 months in 1987, the others received therapy with 2 anti-tuberculosis drugs (HE or SH or SZ) or 3 drugs (SHP or SHE). Similar information was not available for 9 previously treated patients. The treatment of these patients took place in prison and prescribed drugs were unknown. The results of previous treatment for TB were the following. Six of the 30 (20%) patients were cured, 14 patients (46.7%) had treatment failure and 10 patients (33.3%) interrupted previous treatment. Interruption of treatment can be caused by patient and system related reasons. In all 10 cases the previous treatment for TB was interrupted due to patients related reasons, as 5 patients did it due to alcohol intake, 4 patients interrupted treatment according to their own decision and 1 patients ad side effects to anti-tuberculosis drugs.

A total of 36 of the 119 (30.3%) patients interrupted actual treatment for TB. Interruption of treatment can be caused by patient and system related reasons. In all 36 cases the treatment was interrupted due to patients related

reasons (Figure 9). Patient could interrupt treatment because of side effects to anti-tuberculosis drugs, alcohol intake and his own motivation. Seventeen of the 36 (47.2%) patients interrupted treatment due to alcohol intake. Sixteen of the 36 (44.5%) patients interrupted treatment according to their own decision. Three of the 36 (8.3%) patients interrupted treatment because they had side effects to anti-tuberculosis drugs.

Figure 6 Social factors of 119 patients with pulmonary TB

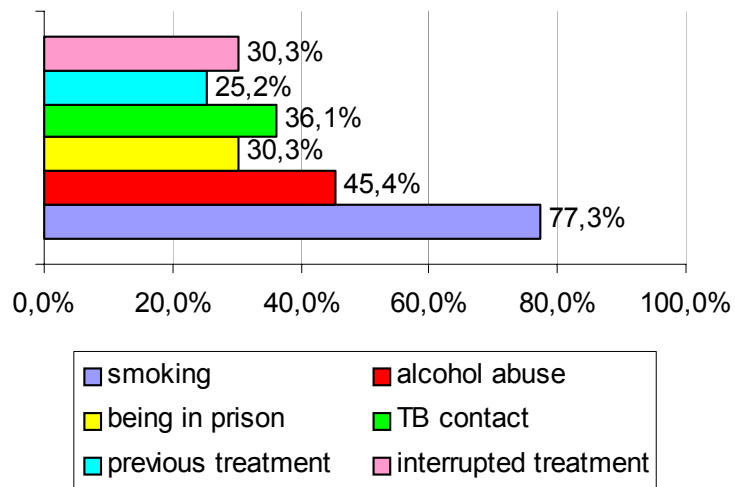


Figure 7 Contact with another TB patient of 43 patients with pulmonary TB

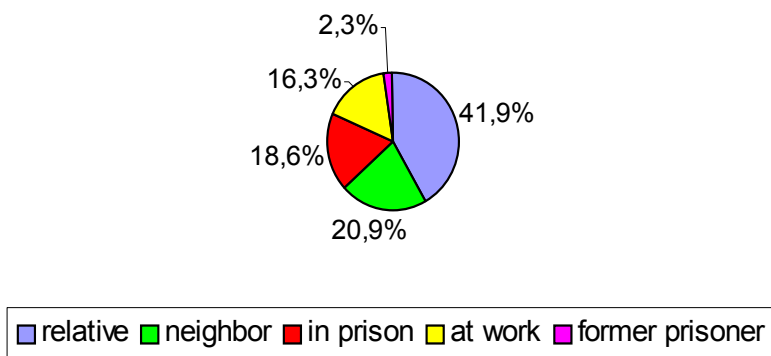


Figure 8 Details of previous treatment of 30 patients with pulmonary TB

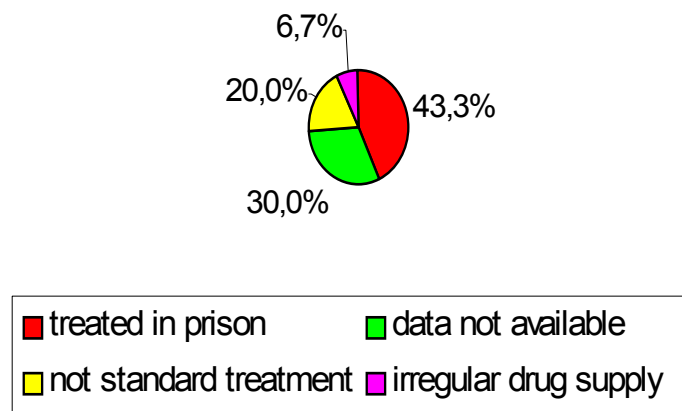
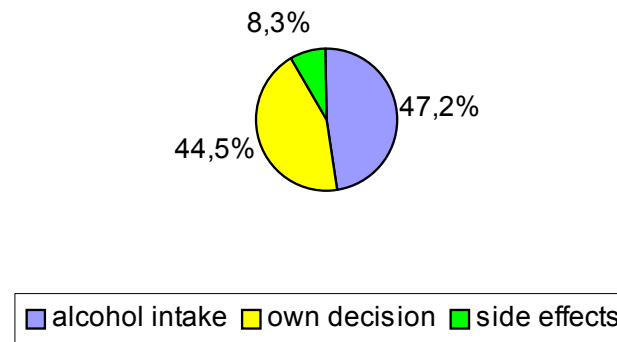


Figure 9 Reasons for interrupted treatment of 36 patients with pulmonary TB



Factors associated with infection with drug-resistant and multi-drug resistant strain of *M. tuberculosis*

To identify factors associated with the development of drug resistance in the Arkhangelsk oblast demographical, social and medical factors collected from the medical records of the ARTD were studied in relation to susceptibility patterns of *M. tuberculosis* strains.

The majority in both groups of patients infected with resistant and susceptible *M. tuberculosis* was males, while there were more female patients infected with resistant strain (34.3%) than infected with susceptible (13.5%) (Table 12). Female gender was significantly associated with infection with drug-resistant strain ($P = 0.01$). Female gender was also significantly associated with infection with multi-drug resistant strain ($P = 0.03$) (Table 13).

Patients previously treated for TB were infected either with resistant or susceptible *M. tuberculosis*. A total of 23 of the 67 (34.3%) patients infected with drug-resistant strain had an evidence of previous treatment for TB. Seven of the 52 (13.5%) patients infected with susceptible strain were treated previously. Previous treatment significantly contributed to development of the disease with drug-resistant strain ($P = 0.01$) (Table 12).

A total of 18 of the 30 (60.0%) patients infected with multi-drug resistant strain had an evidence of previous treatment for TB. Twelve of the 89 (13.5%) patients infected with non-multi-drug resistant strain were treated previously. Previous treatment was significantly associated with disease due to a multi-drug resistant strain ($P < 0.001$) (Table 13).

A total of 27 of the 67 (40.3%) patients infected with drug-resistant strain of *M. tuberculosis* have interrupted treatment for TB, while only 9 of the

52 (17.3%) patients infected with drug-susceptible strain did the same. Interruption of treatment for TB was significantly associated with drug resistance ($P = 0.01$) (Table 12).

A total of 15 of the 30 (50.0%) patients infected with multi-drug resistant strain of *M. tuberculosis* have interrupted treatment for TB, while 21 of the 89 (23.9%) patients infected with non-multi-drug resistant strain did the same. Interruption of treatment for TB was significantly associated with multi-drug resistance ($P < 0.001$) (Table 13).

Patients infected with drug-resistant and susceptible strains did not have significant difference in having COPD and diabetes prior to TB diagnosis. While patients infected with susceptible *M. tuberculosis* were more likely to suffer from gastric ulcer prior to TB diagnosis than those infected with drug-resistant strain ($P = 0.01$) (Table 12).

For the 46 employed patients occupation was classified into work with physical and mental activity. Twenty-four of the 46 (52.1%) employed patients were infected with drug-resistant strain. The remaining 22 of the 46 (47.9%) employed patients were infected with susceptible strain. Eleven of the 24 (45.8%) employed patients infected with resistant strain had a physical work. While 18 of the 22 (81.8%) employed patients infected with susceptible strain had a physical work. Patients having physical work were more likely to be infected with susceptible *M. tuberculosis* ($P = 0.01$) (Table 12). Similar association was observed between having physical work and being infected with non-MDR strain ($P = 0.02$) (Table 13). Patients with mental work had no significant difference in relation to infection with drug-resistant or susceptible strain.

Patients employed in health sector were analysed separately. Five of the 46 employed patients (10.9%) were employed in the health sector of Arkhangelsk as doctors, nurses and nurse assistants. All these patients were infected with drug-resistant *M. tuberculosis*. Three of the 5 patients were new and had MDR. Employment in the health sector of Arkhangelsk was significantly associated with the development of drug resistance ($P = 0.03$).

No significant difference was observed between patients infected with drug-resistant and susceptible *M. tuberculosis* strains in relation to age, BMI, marital status, level of education, crowded accommodation, unemployment, smoking habit, alcohol abuse and contact with another TB patient. Surprisingly an evidence of being in prison was not associated with drug resistance (Table 12) and MDR (Table 13).

Table 12 Demographic and medical profile of 119 patients with pulmonary TB in selection to infection with resistant and susceptible *M. tuberculosis*

Characteristics	Infection with drug-resistant strain n = 67 (%)	Infection with drug-susceptible strain n = 52 (%)	OR (95% CI)	P
Female	23 (34.3)	7 (13.5)	3.4 (1.2 – 9.7)	0.01
Unemployment	31 (46.3)	25 (48.1)	0.9 (0.4 – 2.1)	0.84
Smoking habit	51 (76.1)	41 (78.8)	0.9 (0.3 – 2.2)	0.72
Alcohol abuse	28 (41.8)	26 (50.0)	0.7 (0.3 – 1.6)	0.37
Contact with TB patient	29 (43.3)	14 (26.9)	2.1 (0.9 – 4.9)	0.07
Evidence of being in prison	17 (25.4)	19 (36.5)	0.6 (0.3 – 1.4)	0.18
COPD	17 (25.4)	10 (19.3)	1.4 (0.5 – 3.8)	0.43
Gastric ulcer	1 (1.5)	7 (13.5)	undetermined	0.01
Diabetes	5 (7.5)	2 (3.8)	2.0 (0.3- 16.0)	0.34
Previous treatment for TB	23 (34.3)	7 (13.5)	3.4 (1.2 – 9.7)	0.01
Interruption of treatment for TB	27 (40.3)	9 (17.3)	3.2 (1.2 – 8.3)	0.01

OR = odds ratio; 95%CI = 95% confidence interval; TB = tuberculosis; COPD = chronic obstructive pulmonary disease

Table 13 Demographic and medical profile of 119 patients with pulmonary TB in selection to infection with MDR and non-MDR *M. tuberculosis*

Characteristics	Infection with MDR strain n = 30 (%)	Infection with non-MDR strain n = 89 (%)	OR (95% CI)	P
Female	12 (40.0)	18 (20.2)	2.6 (1.0 – 7.1)	0.03
Unemployment	12 (40.0)	44 (49.4)	0.7 (0.3 – 1.7)	0.37
Smoking habit	20 (66.7)	72 (80.9)	0.5 (0.2 – 1.3)	0.11
Alcohol abuse	12 (40.0)	42 (47.2)	0.8 (0.3 – 1.9)	0.49
Contact with TB patient	14 (46.7)	29 (32.6)	1.8 (0.7 – 4.6)	0.16
Evidence of being in prison	6 (20.0)	30 (33.7)	0.5 (0.2 – 1.5)	0.16
COPD	6 (20.0)	21 (23.6)	0.8 (0.3 – 2.5)	0.68
Gastric ulcer	1 (3.3)	7 (7.9)	0.4 (0.1 – 3.6)	0.35
Diabetes	3 (10.0)	4 (4.5)	2.4 (0.4- 13.8)	0.24
Previous treatment for TB	18 (60.0)	12 (13.5)	9.6 (3.4 – 28.4)	<0.001
Interruption of treatment for TB	15 (50.0)	21 (23.9)	3.2 (1.2 – 8.4)	<0.001

OR = odds ratio; 95%CI = 95% confidence interval; TB = tuberculosis; COPD = chronic obstructive pulmonary disease

CHAPTER IV

DISCUSSION

In the thesis we described the problem of resistance to anti-tuberculosis drugs among new and previously treated patients, the molecular epidemiology of *M. tuberculosis* strains circulating in the Arkhangelsk oblast, Russia, and we identified community factors associated with the development of drug-resistant TB.

The magnitude of M. tuberculosis drug resistance in the Arkhangelsk oblast

The resurgence of TB in the Arkhangelsk oblast during the past 10 years has been accompanied by high rates of drug resistance. Primary and acquired drug resistance in 1991 was 15% and 60%, respectively. In 2000, these parameters increased to 33% and 85%, respectively (unpublished data from the ARTD).

Nowadays, TB and MDR are considered to be synonyms in Russia.⁹ At the same time the actual rates of drug resistance in Russia are not known, as there are no national statistics available.^{9,16} A major challenge nowadays is to monitor and control resistance to anti-tuberculosis drugs. A broad range of resistance rates has been reported by different oblasts, but overall drug-resistant TB is common. According to surveys presented in the literature, primary and acquired MDR ranged from 4.0 to 14.4% and from 19.2 to 54.4%, respectively.²⁰ In the Ivanovo oblast, 5% of the strains collected from civilian patients with new cases of pulmonary TB during 1995-1996 were multi-drug

resistant.⁵² Resistance to at least one drug among new and previously treated patients were 29.0-32.4% and 57.8-68.5%, respectively.²²

Our study shows that resistance to at least one drug among new and previously treated cases was 49.4% and 76.7%, respectively. MDR among new and previously treated cases was found to be 13.5% and 60.0%, respectively. Drug resistance rates in the Arkhangelsk oblast were thus higher than those described for different oblasts of Russia.

In our study, monoresistance was found at very low rates for S, H and E. At the same time resistance to two, three and four drugs was observed more often. That is in agreement with another study from Russia.¹⁸ The fact that the highest rates of resistance in the Arkhangelsk oblast were observed for S and H was not surprising. As it was reported before, primary resistance to S and H was 40.0% and 25.0%, respectively,⁷⁵ and parameters of acquired resistance were 84.0% and 82.0%, respectively. In our study, parameters of resistance among new cases are similar to those described before. The rates of resistance among previously treated cases have decreased.

We did not observe monoresistance to R. Resistance to R predicted resistance to H and served as a marker of MDR. The fact that resistance to R is rarely observed without associated resistance to other drugs was confirmed by other studies.^{37,76}

Transmission of drug-resistant M. tuberculosis strains

High rates of resistance to anti-tuberculosis drugs among new cases indicate that drug-resistant strains are circulated and transmitted from patient to patient in a high degree in the Arkhangelsk oblast. Transmission of already

resistant strains is a very important problem, as it impacts case management. TB control programme should have reliable laboratory facilities to diagnose drug resistance. The spread of multi-drug resistant strains raises the necessity to implement rapid diagnostic methods for detection of resistance like Inno-Lipa.

Active transmission of *M. tuberculosis* resistant strains in the community can be measured. It is generally assumed that the proportion of clustered strains in a population reflects the amount of recent transmission.⁷⁸ In our study, only 15.7% of *M. tuberculosis* strains were clustered. It was impossible to establish connections between all patients included in the same cluster. At the same time, it is evident that clustered cases belonged to the same chain of transmission directly or indirectly. The largest cluster in our material comprised 14 patients. Studies performed in Russia using RFLP analysis have revealed that the majority of clusters were small with 2 to 4 patients per cluster.^{77,79} In the study conducted in the North-West part of Russia the maximum cluster size consisted of 10 patients.⁸⁰ RFLP clusters contained 2, 3, 5 and 8 strains in the studies performed in Asian countries on *M. tuberculosis* strains isolated from patients with pulmonary TB.^{81,82} The sample size of these studies were several times higher than ours. Although the proportion of strains in clusters was relatively low, a large size of RFLP clusters indicates existence of active transmission of *M. tuberculosis* strains in the Arkhangelsk oblast.

M. tuberculosis strains included in the same RFLP cluster have different mutations in the *rpoB* gene and, in addition, had resistance to various other drugs. This suggests active transmission of both drug-resistant and drug-susceptible strains of *M. tuberculosis*.

Mutations responsible for R resistance

Our study shows that *M. tuberculosis* strains resistant to R have different mutations in the *rpoB* gene. The fact that R5 pattern was the most common corresponds with previously published data.^{37,71-74,77} One strain was represented by two subpopulations of bacilli, one susceptible and one resistant to R. The mutation responsible for R resistance could have occurred during treatment, as the strain was isolated from patient previously treated for TB. The population of susceptible bacilli was not totally substituted by the population of resistant bacilli at the time of strain isolation. Determination of mixtures of resistant and susceptible bacilli, especially mixture of WT *M. tuberculosis* strain and resistant having R4_a pattern was described before.^{37,66}

Analysis of epidemiological clusters

Patients with TB included in the epidemiological clusters presented the most economically productive age group, as their mean age varied from 30.0 to 43.3. The majority of patients within the same cluster were males. The fact that a small proportion of patients (9.1%-21.4%) within the same epidemiological cluster have moved to the Arkhangelsk oblast for less than 10 years ago indicates that migration in the Arkhangelsk oblast is low. TB epidemiological situation there is not influenced by the process of migration.

Every epidemiological cluster contained patients from the city of Arkhangelsk and from different parts of the oblast as well. Although it was practically impossible to establish all connections between patients included in the same cluster, it is likely that these patients belonged to the same chain of TB transmission, indicating the transmission is not limited in the city.

Risk factors for the development of drug-resistant TB

Identification of factors associated with the development of drug resistance is very important and helpful in planning anti-tuberculosis activities. A case control study conducted in the Ivanovo oblast, Russia, found that only homelessness was a risk factor for primary drug resistance.⁷⁴ This factor was not relevant for the Arkhangelsk oblast, as only 2 patients of the total 119 were homeless. In the Tomsk oblast, the major factor associated with MDR was higher socio-economic status, implying access to health services/anti-tuberculosis medications and ongoing transmission, although these cases denied prior use of anti-tuberculosis drugs.⁹ Access to health services and contact with infectious TB cases was frequent for our 5 patients employed in the health sector. Three of these 5 patients were newly diagnosed and they were infected with multi-drug resistant *M. tuberculosis*. The fact that all these patients were infected with resistant strains deserves special attention.

All patients included in our study were HIV negative. Increase of TB morbidity and mortality rates observed during the past 10 years in Russia was not associated with the HIV epidemic.²⁰

In the Arkhangelsk oblast, female gender was significantly associated with the development of drug resistance even though the majority of the patients were male. The same was described in Estonia, previous state of the Soviet Union.⁸³ The opposite phenomenon is observed in United Kingdom where male gender is considered to be risk factor for drug-resistant TB.

Certain social factors such as smoking habit, alcohol abuse and unemployment were not associated with the development of drug resistance in the Arkhangelsk oblast, although found by others.^{52,83}

The surprising factor that a history of being in prison in spite of a significantly higher rates of drug resistance in the prison system of Russia was not associated with resistance to anti-tuberculosis drugs was also described in the Ivanovo oblast.⁵² Detailed analysis allowed to reveal that 21 of the 36 patients who have had a history of being in prison (58.3%) were released from the prison during the 1970s and 1980s. While the epidemic of TB in the prison system of the Arkhangelsk oblast has been observed since 1994.²¹ Sixteen of the 36 previous prisoners (44.4%) have been treated for TB. The present illness of these patients could be due to reactivation of the infection (probably drug sensitive) that they have got while being in prison 20-30 years ago. At the same time these patients could be re-infected after the release from prison. In this case, they had a chance of being infected with either resistant or susceptible strain. It is generally believed that, if the prevalence of TB in the community is very low, TB is mainly caused by endogenous reactivation of primary infection.⁸⁴ On the other hand, TB among people living in high TB endemic areas is assumed to be caused mainly by re-infection with *M. tuberculosis*.⁸⁴ The risk of the disease development is influenced by the epidemiological situation in the community. At the same time there is no clear evidence confirming that exogenous re-infection is the predominant cause of TB in high endemic countries.^{85,86}

A previous treatment for TB was defined as treatment with anti-tuberculosis drugs documented in the medical records of the ARTD. Evidence of previous treatment for TB was significantly associated with the development resistance to anti-tuberculosis drugs. This is in agreement with previously published data.^{25,34,42,45,87}

Poor patient compliance is the most important reason for the development of drug-resistant TB. Interruption of treatment for TB can be due to patient and system related reasons. Our study has shown that patient related reasons for non-compliance were common in the Arkhangelsk oblast.

Future perspectives

The results of present study can serve as a helpful tool for planning further anti-tuberculosis activities in the Arkhangelsk oblast aimed to control the spread of drug-resistant TB. Treatment based on different case categories administered under direct supervision is very useful to prevent the development of drug resistance during treatment. The implementation of treatment based on the DOTS strategy in the Arkhangelsk oblast was started in 1998. Medical staff was educated in new strategy. The treatment for TB is prescribed according to different categories. Every case is discussed at the central doctor's commission at the time of diagnosis and end of intensive phase of treatment. Special attention is paid to the patients infected with drug-resistant *M. tuberculosis*. Treatment of these cases is prescribed according to the individual susceptibility pattern, following the guidelines.⁵⁹ All this is done in order to avoid mistakes in case management. Treatment of patients infected with susceptible *M. tuberculosis* strains prescribed according to standard treatment regimens under direct supervision should led to cure. Administration of treatment to new patients infected with drug-resistant strains is problematic. Usually, 10-12 weeks will pass before the results of susceptibility test will be available. Treatment prescribed to a patient with a new case of pulmonary TB according to the category I can be equivalent of monotherapy if

a strain is resistant to 3 drugs. All these raises importance of diagnosis of drug resistance on time and importance of proper treatment regimen.

Poor patient's compliance is of great importance as interruption of treatment is the main reason for accumulation of mutations responsible for resistance to anti-tuberculosis drugs. Unfortunately, in Russia there is no law concerning TB treatment, in contrast to many countries of Western Europe where the law states that individual suffering from infectious TB creates risk for the health of other citizens and is obliged to be treated. Patients with poor compliance create conditions for the spread of *M. tuberculosis* in the community, especially patients having a drug-resistant TB creates a severe risk for the health of many people. Infection with drug-resistant strain can result in more serious disease and necessity to prescribe second line drugs that are less effective and have more side effects. Our study has shown that non-compliance in the Arkhangelsk oblast is due to patients related reasons. Establishment of a law on TB treatment with definition of patient's and doctor's responsibilities can help to solve the problem.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. Drug-resistant TB is an important problem in the Arkhangelsk oblast, Russia.
2. Mono-resistance to anti-tuberculosis drugs is a rare phenomenon to observe. Simultaneous resistance to two or three drugs is common phenomenon.
3. MDR exists at an alarming level in the Arkhangelsk oblast.
4. Resistance to R predicts resistance to H and can serve as a valid marker of MDR in the Arkhangelsk oblast.
5. The highest rates of resistance are observed for S and H.
6. Drug-resistant strains of *M. tuberculosis* are circulating in all raions of the Arkhangelsk oblast and they are transmitted at a high degree.
7. The spread of drug-resistant TB is attributed to several risk factors: being female, evidence of previous or interrupted treatment for TB are the risk factors for the development of drug-resistant TB in the Arkhangelsk oblast.
8. Employment in the health sector of Arkhangelsk was significantly associated with the development of drug resistance.

Recommendations

1. It is important to monitor and control resistance to anti-tuberculosis drugs.
2. The spread of drug-resistant TB raises the necessity to implement reliable laboratory facilities to diagnose drug resistance on time. Rapid methods of diagnosis of resistance to R, for example with Inno-Lipa, can serve as a helpful tool.
3. Treatment of TB based on different case categories administered under direct supervision is useful to prevent non-compliance and development of drug resistance.
4. TB control programme should use all efforts to ensure proper treatment of TB patients. Establishment of law on TB treatment can help to solve the problem.
5. Special attention should be paid and preventive measures undertaken in order to control the development of TB with drug-resistant *M. tuberculosis* among medical personnel.

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APPENDIX
DATA COLLECTION FORM

Patient information

First name, family name _____

Culture number (ARTD) _____ NIPH number _____

Date of registration _____

Gender: male, female

Date of birth _____ age _____

Weight _____ kg

Height _____ cm

BMI _____

Address (raion) _____

Has been living in the Arkhangelsk oblast for _____ years

Marital status: married, single, divorced, widowed

Social information

Education: primary, secondary, higher

Occupation: student

employed: industrial worker, office staff, medical staff

unemployed

retired

Housing: non, hostel, flat, house

Number of rooms _____ number of persons living together _____ number of children under age of 15 _____ number of persons per room _____

Smoking: no, yes

Narcotics abuse: no, yes

Alcohol abuse: no, yes

TB among relatives or other persons living together with the patient:

no, yes

Being in prison: no, yes, for how many years _____, when _____

Medical information

COPD: no, yes: bronchial asthma

pneumonia more than twice

chronic bronchitis

Diabetes: no, yes

Peptic ulcer: no, yes

HIV status: negative

positive

Case category: New case

Previously treated case

For patients treated previously:

Year when treatment took place _____

Prescribed drugs during the previous treatment _____

Previous treatment took place in prison: yes, no

Previous treatment was prescribed not according to the standard regimens for different case categories: no, yes

Irregular drug supply during treatment: no, yes

Results of treatment: cured, treatment completed, defaulted, failure,
 relapse, chronic case, transferred out

Laboratory data:

1. Smear microscopy: date _____ positive, negative
2. Culture date _____

Present treatment:

Date of prescription _____

Prescribed drugs _____

Alcohol intake during treatment: no, yes

Interruption of present treatment: no, yes

Reasons for interruption of present treatment:

system related (irregular drug supply)

patient's related (non-compliance with hospital rules, patient's decision)

Side effects of drugs: no, yes

Microbiology data

Results of susceptibility test (BACTEC) performed at the NIPH:

E: susceptible, resistant

H: susceptible, resistant

R: susceptible, resistant

S: susceptible, resistant

Any resistance: no, yes

MDR: no, yes

Inno-Lipa for R resistant strains only: susceptible, resistant

Inno-Lipa pattern_____

RFLP:

Clustered strain: no, yes

Number of IS6110 copies_____