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EVALUATION OF DETECTION AND DRUG RESISTANCE OF MYCOBACTERIUM TUBERCULOSIS IN PATIENTS IN THE ŁÓDZKIE VOIVODSHIP IN 2009-2013

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ABSTRACT

STUDY AIM. Evaluation of detection and drug resistance of *Mycobacterium tuberculosis* in patients from the Łódzkie Voivodship in the period 2009 – 2013.

MATERIAL AND METHODS. The data presented in the study include information obtained while diagnosing patients from the Łódzkie Voivodship in order to detect infections with *Mycobacterium tuberculosis* in the period 2009 – 2013.

RESULTS. In 2009 – 2013 we analyzed clinical specimens for the purpose of detection of *Mycobacterium tuberculosis*. Tubercle bacilli were confirmed in 5621 specimens in 2196 patients; positive bacterioscopy results were observed in 1724 clinical specimens. In the study period 18 clinical specimens obtained from children contained tubercle bacilli. In the period 2009 – 2013 we noted multi-drug resistant (MDR) strain in 41 clinical specimens, which made up 1.8 % of strains with known results of drug-sensitivity. In 5 clinical specimens we observed extensively-drug resistant (XDR) strain, which made up 0.2 % of strains with known results of drug-sensitiveness. 12 clinical specimens appeared to contain pre-XDR strain, which constituted 0.6 % of strains with known results of drug-sensitivity.

SUMMARY AND CONCLUSIONS. Despite advances in the diagnostics and treatment of tuberculosis (TB) this diseases still poses a serious medical problem. The detection level in the period 2009 – 2013 is relatively unchanged, with regards to both bacterioscopy and culture methods. Thus, the laboratory detection of tuberculosis bacilli is similar. It directly results from the enforcement of strict procedures regarding the quality of specimens collected for microbiological purposes and the control of the performed tests, which contributes to a greater number of confirmed cases of TB. In the study period the number of new cases of the infectious diseases is variable. Only in children this number remains stable over the years. Researchers observe that tubercle bacilli are resistant to basic first-line treatment drugs. They also note the occurrence of MDR, pre-XDR and XDR strains. Hence, it is important to regularly and carefully monitor the sensitivity of *Mycobacterium tuberculosis* to antibiotics administered in a long-term anti-tuberculosis therapy.

Key words: *Mycobacterium tuberculosis*, *TB, bacteriological confirmation, drug-resistance*

INTRODUCTION

Tuberculosis is a contagious disease caused by intracellular pathogens, bacilli of *Mycobacterium tuberculosis*. It usually develops in lungs but it can also occur in other organs (extrapulmonary tuberculosis). It is worth pointing out that a relatively small percentage of people infected with the bacteria develop full-blown tuberculosis. TB is the second most serious contagious disease. It follows the human immunodeficiency virus (HIV), which contributes to the highest number of deaths due to contagious diseases all around the world.

According to WHO estimates, 9 million people were infected with TB and of that number 1.5 million died. It is estimated that mainly due to making proper diagnoses and introducing effective therapies in 2000 – 2013 it was possible to prevent 37 million people from death (1, 2).

A crucial breakthrough in combating TB occurred in the second part of the 20th century after discovering many anti-tuberculosis drugs and introducing them into

therapies. Within two years following the introduction of streptomycin in 1943 and rifampicin in 1963 into therapies a significant decrease in the death rate due to TB was observed and the spread of tubercle bacilli was substantially reduced. Current treatment of drug-resistant TB involves a six-month application of four first-line anti-tuberculosis drugs: isonizaid, rifampicin, ethambutol, and pyrazinamide. Unfortunately, improper application of anti-tuberculosis drugs in the 1980s resulted in the occurrence of strains which do not respond to treatment, so called MDR (*Multi-Drug Resistans*). Treatment of this type of TB takes more time (about 20 months). It requires administering more expensive and more toxic drugs and it might be less effective.

MICROBIOLOGICAL METHODS OF DIAGNOSING TUBERCULOSIS

Microbiological tests (microscopic tests and culture on diagnostics media) are a referential method for detection of TB and are performed if any cases of the disease are suspected. In order to diagnose TB a lot of methods of various sensitivity and specificity is use and in which they obtain results after various periods of time. It must be pointed out that bacteriological tests for TB can be performed only in laboratories supervised by the Polish National Reference Tuberculosis Laboratory. The Laboratory for Diagnostics of Tuberculosis – Medical Laboratory, dr n.med. Teresa Fryda Sp. z o.o. at the Provincial Health Care Centre Network, the Centre for the Treatment of Lung Diseases and Medical Rehabilitation in Lodz meets the above requirements (3). The first and the most basic method for diagnosing TB is bacterioscopy of smears from samples obtained from a patient (except for urine), where, under a microscope, the bacteria stained with the application of the Ziehl-Neelsena method are observed. Further selection of diagnostic methods depends on the result of the bacterioscopy test (3, 4). Its result can be a ground neither for confirming nor for ruling out TB because positive smear can contain dead tubercle bacilli or MOTT bacilli (*Mycobacterium other than tuberculosis*). Thus, in order to confirm bacilli, a culture test on solid and liquid media is performed. After obtaining results of the bacteriological test the cultured bacilli are identified each time and a drug-resistance test is performed. The molecular tests with the application of probes specific for *Mycobacterium tuberculosis* are also carried out. Despite their high specificity and sensitivity genetic methods cannot replace the traditional culture method, which is a reference method. They only serve as supplementary methods. In laboratories mycobacterioses induced by MOTT bacilli are also diagnosed. They are cultivated on media used for growing tubercle bacilli. Thus, in each culture, it is essential to differentiate *Mycobacterium tuberculosis complex* from MOTT.

An integral procedure in the diagnostics of TB, after tubercle bacilli have been confirmed in the culture, is a test, evaluating the drug-resistance to four basic drugs, i.e. isonizaid (INH), streptomycin (SM), rifampicin (RMP) and PZA in determining of drug resistance by automated methods, ethambutol (EMB) (in all new tuberculosis patients who developed bacilli) and to other drugs, if the patients have appeared to demonstrate MDR or XDR resistance (5).

In most cases the occurrence of the disease can be clinically confirmed with laboratory tests. The percentage is lower for bacterioscopy (around 40%); with regards to inoculation it is about 70%. In the year 2008 in 40.7% of cases of pulmonary tuberculosis the results of the bacterioscopy tests turned out positive (6). In 2009 such results were observed in 40.0% cases (7), in 2010 - in 40.1% (8), in 2011 – in 37% (9), in 2012 - in 39.6% (10), in 2013 – 41.8% (11). In 2008 the occurrence of pulmonary tuberculosis was confirmed in bacteriological tests in 65.4% of cases (6), in 2009 the result was positive in 65.8% (7), in 2010 – in 65.6% (8), in 2011 – in 67.6% (bacteriological confirmation of the incidence of pulmonary tuberculosis 13.9) (9), in 2012 – in 69.4%, in 2013 – 68.2% (11). In 2008 the incidence of bacteriologically confirmed TB was 13.4 per 100,000 population. In subsequent years (2009 – 2013) the incidence rate of bacteriologically confirmed TB fluctuated significantly: +2.23% (2008 *vs.* 2009); -8.76% (2009 *vs.* 2010); +16.80% (2010 *vs.* 2011); -9.59% (2011 *vs.* 2012); -5.30% (2012 *vs.* 2013). In a diagnostic process in 2013 the incidence rate of bacteriologically confirmed TB was 12.5, which made up 66.6% of the total number of patients.

TB therapy has to be conducted in compliance with certain guidelines. These procedures should be observed prior to the initiation of the therapy as well as during the therapy. In order to bacteriologically confirm the infection with tubercle bacilli it is recommended to perform a smear test and prepare a culture test from three consecutive sputum specimens collected from a patient, at best, on three consecutive days. Both the stages of the therapy, the intensive one and the eradicating treatment are regularly monitored bacteriologically. In the event of new cases of TB the first test is conducted prior to the commencement of the therapy. Patients with pulmonary tuberculosis caused by bacilli resistant to basic drugs undergo a bacterioscopy test and a sputum culture two months after the commencement of the treatment. After three months of the treatment the bacteriological test is conducted again. If the patient still demonstrates active TB, the drug-sensitivity is re-evaluated. A final test is carried out after completion of the therapy, i.e. after 6 months. If pulmonary tuberculosis has been caused by multi-drug resistant bacilli, a bacterioscopy test

and a sputum culture are performed every month until the disease turns into non-active and then, every three months until the completion of the therapy. Previously treated patients, who are being administered another TB therapy, are supposed to have bacteriological tests done at the end of the 3rd, 5th, and 8th month of the therapy. It must be emphasized that while treating the patient again, one should not use the resistance profile applied in the previous episode of the infection (4, 5).

EPIDEMIOLOGY OF TUBERCULOSIS IN POLAND

The Polish legislature has introduced a lot of regulations which enable to monitor TB. According to the Act of 5 December 2008 on prevention and control of infections and infectious diseases in humans, the Act of 13 July 2012 amending the above Act and the Act on the State Sanitary Inspection, doctors are required to report all cases of TB within 24 hours following the detection. The cases are first reported to district and then to provincial sanitary and epidemiological stations and these send quarterly reports to the National Tuberculosis and Lung Diseases Research Institute. In cooperation with the Chief Sanitary Inspectorate the institute prepares reports on the number of cases of TB registered in Poland. The National TB Register has been kept in the institute, in the Department of Tuberculosis Epidemiology and Surveillance for 55 years.

The Laboratory for Diagnostics of Tuberculosis – Medical Laboratory, dr n.med. Teresa Fryda Sp. z o.o. at the Provincial Health Care Centre Network, the Centre for the Treatment of Lung Diseases and Medical Rehabilitation in Lodz plays an important laboratory role by monitoring TB in the Łódzkie Voivodship. The laboratory is required to report any occurrence of tubercle bacilli detected in samples obtained from a patient to the Provincial Sanitary and Epidemiological Station. The procedure applies to each new case of infection, an infection which re-occurred and the occurrence of multi-drug resistant strains.

A new case of an infection refers to patients who were not administered an anti-tuberculosis therapy for longer than one month (2). In 2008 new cases of the disease made (7) up 87.3% of all registered cases (6), in 2009 – 88.2% (7) in 2010 – 88.0% (8), in 2011 – 88.6% (9), in 2012 – 88.4 %, in 2013 – 88.3% (11). The Laboratory Studio for Diagnostics of Tuberculosis – Medical Laboratory, dr n.med. Teresa Fryda Sp. z o.o. at the Provincial Health Care Centre Network, the Centre for the Treatment of Lung Diseases and Medical Rehabilitation in Lodz reports cases of an infection detected in all hospitals, outpatient clinics and prisons in the Łódzkie Voivodship. Obtained results allow to report all new cases of TB in the region.

In 2008 the incidence of TB (all types) in Poland was 21.2 per 100,000 population. In subsequent years (2009 – 2013) the incidence rate fluctuated significantly: +1.88% (2008 *vs.* 2009); -8.79% (2009 *vs.* 2010); +12.69% (2010 *vs.* 2011); -11.71% (2011 *vs.* 2012); -4.08% (2012 *vs.* 2013). In the above period we observed an average decrease in the incidence of TB, i.e. 2%. In 2013 the incidence rate for TB was significantly different in different voivodships. Figure 1 presents current data on the incidence of TB in Poland in 2001 – 2012. The most serious epidemiological situation was observed in the Lubelskie voivodship (2013 – 27.4 per 100,000 population), the Świętokrzyskie voivodship (2013 – 24.3 per 100,000 population) and the Śląskie voivodship (2013 – 23.9 per 100,000 population). Despite the above fact, we have been observing a constant decrease in the number of TB cases in Poland for a decade. The lowest incidence of TB was noted in the Wielkopolskie voivodship (2013 – 9.9 per 100,000 population), the Warmińsko-mazurskie voivodship (2013 – 13.3 per 100,000 population), the Podlaskie voivodship (2013 – 12.5 per 100,000 population), the Podkarpackie voivodship (2013 – 13.1 per 100,000 population). Table 1 presents the incidence rate of TB, including the rate for the Łódzkie voivodship.

MATERIAL AND METHODS

The first and the most basic method for diagnosing TB is bacterioscopy of smears from samples obtained from a patient (except for urine), where, under a microscope, the bacteria stained with the application of the Ziehl-Neelsena method were observed. Further selection of diagnostic methods depends on the result of the bacterioscopy test [3, 4]. It should be pointed out that its result can be a ground neither for confirming nor for ruling out TB because the positive smear can contain dead tubercle bacilli or MOTT bacilli (*Mycobacterium other than tuberculosis*).

Bacteriological methods, apart from a microscopic examination, also include culture on diagnostic media. They are a referential method for detection of TB and are performed if any cases of the disease are suspected. For the purpose of detecting tubercle bacilli, in the Laboratory for Diagnostics of Tuberculosis – Medical Laboratory, we applied two methods, as recommended by WHO; the first one was a conventional (traditional) method, i.e. inoculation on the Löewenstein-Jensen and Stonenbrink solid media and the other one was an automatic (fluorescence) method, i.e. inoculation on the Middlebrook media with the application of the MGIT

Table I. Incidence of tuberculosis per 100,000 population in 2009 - 2013. Comparison of the Łódzkie voivodship with the whole population of Poland

Incidence rate		Poland					Łódzkie voivodship				
		2009	**2010**	**2011**	**2012**	**2013**	**2009**	**2010**	**2011**	**2012**	**2013**
Incidence in total	In total	21.6	19.7	22.2	19.6	18.8	29.4	31.9	29.4	28.1	22.8
	Pulmonary tuberculosis	20.1	18.3	20.5	18.2	17.8	28.0	30.5	28.2	26.2	22.0
	Extrapulmonary tuberculosis	1.5	1.4	1.6	1.4	1.1	1.4	1.4	1.2	1.9	0.8
Incidence confirmed microbiologically	In total	13.7	12.5	14.6	13.2	12.5	14.9	13.3	16.6	17.1	13.5
	Pulmonary tuberculosis	13.2	12.0	13.9	12.6	12.1	14.4	13.0	16.1	16.3	13.3
	Extrapulmonary tuberculosis	0.5	0.4	0.7	0.5	0.4	0.5	0.2	0.5	0.8	0.2

960 automated system (Becton Dickinson). The cultivated bacterial strains were classified into the proper group: TB complex (*Mycobacterium tuberculosis*) and MOTT (*Mycobacterium other than tuberculosis*).

In order to identify the culture we performed two tests: niacin test for cultures grown on solid media or MGIT identification test for cultures grown on solid and liquid media. An integral procedure in the diagnostics of TB, after tubercle bacilli have been confirmed in the culture, is a test, evaluating the drug-resistance to four basic drugs, i.e. INH, SM, EMB, RMP and PZA in determining of drug resistance by automated methods ethambutol (EMB) (in all new tuberculosis patients who developed bacilli) and to other drugs, if the patients have appeared to demonstrate MDR or XDR resistance. Thus, in further stages of the diagnostics, drug-resistance tests with the application of the traditional method on the Löewenstein-Jensen solid media, for evaluation of the resistance to first-line anti-tuberculosis drugs (INH, SM, EMB, RMP) were performed. With regards to MDR strains the resistance to second-line anti-tuberculosis drugs (cycloserine, capreomycin, para-aminosalicylic acid, ofloxacin) and third-line anti-tuberculosis drugs (amikacin, trimethoprin, sulfamethoxazole, clofazimine, erythromycin) was also evaluated. For strains cultured with the fluorescence method drug-resistance tests with the application of the automatic method on the Middlebrook liquid media in the BACTEC MGIT 960 apparatus were performed. The test allowed to evaluate resistance to the following drugs: isonizaid, streptomycin, ethambutol, rifampicin, pyrazinamide.

In order to bacteriologically confirm the infection with tubercle bacilli a smear test and prepared a culture test from at least three consecutive sputum specimens collected from a patient, on three consecutive days were performed. Moreover, in the stage of intensive treatment a microbiological examination was carried out, as ordered by the doctor in charge. The number of ordered tests correlates with the degree of the disease and clinical observation. With regards to patients with highly active TB the number of specimens sent for analysis is bigger. On average, it is usually 5 – 10 samples.

After completing the treatment a microbiological test was we performed twice in order to confirm the eradication of the patients and the effectiveness of the therapy. A final test is carried out after completion of the therapy, i.e. after 6 months for bacilli resistant to basic drugs.

RESULTS

DETECTION OF ACID-FAST BACILLI

Types of studied samples. The most common clinical sample sent to analysis for the diagnostics of *Mycobacterium tuberculosis* was sputum, then bronchoaspirate, pleural fluid, laryngeal swab, gastric washings and urine. Other specimen constituted 1.1% of all the studied specimens. They included: bioptates, peritoneal fluid, cerebrospinal fluid, fistula swab, ear swab, scrapings from the uterine cavity.

Bacterioscopy results. We obtained positive results in 1724 clinical specimens, which confirmed the occurrence of acid-fast bacilli. In 2009 we received 319 positive bacterioscopy results, in 2010 – 251, in 2011 – 300, in 2012 – 416, in 2013 – 438. Positive results in cultures were observed in 28.52% of all the studied specimens. In 2009 positive bacterioscopy results were noted in 28.15% of samples, in 2010 - in 21.99%, in 2011 – in 21.97%, in 2012 - 30.99%, in 2013 - in 41.16% of samples.

Bacteriological confirmation of tubercle bacilli. We bacteriologically confirmed the occurrence of *Mycobacterium tuberculosis* in 5621 studied clinical specimens, in 2196 patients. In 2009 we obtained 1133 positive cultures of acid-fast bacilli. This number included 1096 tubercle bacilli isolated from 451 adult patients and 3 children. In 2010 we obtained positive cultures of acid-fast bacilli. This number included 1082 tubercle bacilli

Table II. Summary of the results obtained from growth and bacterioscopy of acid-fast bacilli in 2009-2013

	2009	2010	2011	2012	2013
% positive smear	1.5	1.3	1.4	2.0	2.6
% positive culture	5.8	5.5	6.1	6.4	6.2
% cultivated tubercle bacilli	96.7	94.8	92.6	90.8	90.3

isolated from 412 adult patients and 3 children. In 2011 we obtained 1365 positive cultures of acid-fast bacilli. This number included 1264 tubercle bacilli isolated from 541 adult patients and 3 children. In 2012 we obtained 1342 positive cultures of acid-fast bacilli. This number included 1218 tubercle bacilli isolated from 473 adult patients and 3 children. In 2013 we obtained 1064 positive cultures of acid-fast bacilli. This number included 961 tubercle bacilli isolated from 319 adult patients and 6 children (Table II).

New cases of TB in the Łódzkie voivodship in 2009 – 2013. In the period 2009 – 2013 we noted 2070 new cases of TB. In 2009 we bacteriologically confirmed the occurrence of 400 new cases of tuberculosis, in 2010 – 383, in 2011 – 516, in 2012 – 473, in 2013 – 298. In 2009 – 2013 the number of new cases of infection with *Mycobacterium tuberculosis* fluctuated: -4.25% (2009 *vs.* 2010); +34.7% (2010 *vs.* 2011); -8.3% (2011 *vs.* 2012); -58.7% (2012 *vs.* 2013).

DRUG-RESISTANCE

The number of tubercle bacilli sensitive to all anti-tuberculosis first-line drugs made up 93.11%. In 2009 we confirmed that 417 strains were sensitive to all anti-tuberculosis first-line drugs. In 2010 – there were 383 such strains, in 2011 – 504 strains, in 2012 – 444 strains and in 2013 – 297 strains. In 2009 – 2013 we isolated MDR strains (resistance to INH + RMP, also in combination with resistance to other drugs) from 41 clinical specimens, which made up 1.8% of strains with known results of drug-sensitivity. In 2009 we isolated 5 MDR strains, in 2010 - 8 strains, in 2011 - 11 strains, in 2012 - 12 strains, in 2013 - 5 strains. In the analyzed period we detected XDR strain (resistance to MDR + fluoroquinolone, amikacin, kanamycin, capreomycin) in 5 clinical specimens, which made up 0.2% of strains with known drug-sensitivity results. In 2009 we isolated 2 XDR strains, in 2011 – 1 strain, in 2012 – 2 strains. In 2010 and 2013 we did not isolate any strains. 12 clinical specimens appeared to contain pre-XDR strain, which made up 0.6% strains with known results of drug-sensitivity. In 2010 we isolated 4 pre-XDR strains, in 2011- 3 pre-XDR strains, in 2012 - 2 pre-XDR strains, in 2013 – 3 pre-XDR strains. In 2009 we did not isolate any pre-XDR strains. Table III presents detailed information on drug-resistance of the isolated tubercle bacillus.

DISCUSSION

Although the number of microbiologically confirmed cases of TB has increased the detection level in various regions in Poland is much different. The percentage of patients with pulmonary tuberculosis confirmed in bacteriological tests was 65.4% in 2008 and ranged from 54.2% to 91.8% (6), in 2009 it was 65.8% and in 40% of all TB patients tubercle bacilli were detected as early as in bacterioscopy. The percentage of patients who were diagnosed with TB after performing a sputum culture ranged from 48.3% to 85.6% (7). In 2010 there were still differences among voivodships with regards to pulmonary tuberculosis. The percentage of bacteriologically confirmed cases ranged from 57.6% to 90.4% (8). Similar differences among voivodships were observed in 2011 and the percentage ranged from 57.2% to 90.0 (9). In 2012 it ranged from 60.8% to 89.7% (10) and in 2013 - from 60.4% to 90.5% (11). Such huge differences might result from a poor quality of bacteriological tests in some areas and a improper diagnosis of active tuberculosis (7). In the study period, positive results of culture obtained in the Laboratory for Diagnostics of Tuberculosis were noted in almost 90% of cases, which is a high percentage if we take

Table III. Drug resistance of *Mycobacterium tuberculosis* isolated from clinical samples

	2009	2010	2011	2012	2013
% strains sensitive to first-line drugs	92.5	93.0	93.2	93.9	93.1
% strains resistant to INH	2.7	3.6	1.3	1.3	1.9
% strains resistant to SM	2.9	1.0	1.8	1.1	2.5
% strains resistant to EMB	0	0	0	0	0
% strains resistant to RMP	0	0.2	0.4	0.4	0.3
% strains resistant to INH+SM	0.9	0.4	0.5	0.8	0.9
% MDR	1.1	1.9	2.0	2.5	1.6
% XDR	0.4	0	0.2	0.4	0
% pre-XDR	0	1.0	0.5	0.4	0.9

the whole country into consideration. Moreover, since 2009 the number of bacteriologically confirmed TB cases, i.e. with positive culture results, has been stable. A similar percentage of bacteriologically confirmed TB cases can be also observed in the European Union (6). With regards to positive results of sputum culture there are huge differences in Poland, too. In the study period positive results of culture obtained in the Laboratory for Diagnostics of Tuberculosis were noted in 28.52% of all analyzed clinical specimens. Concluding, the enforcement of strict procedures regarding the quality of specimens collected for microbiological purposes and conducted tests as well as a better control of the tests result in the stable percentage of the detected and confirmed occurrence of tubercle bacilli in the Laboratory for Diagnostics of Tuberculosis. Besides, an implementation of molecular biology methods will reduce the time needed for obtaining results and increase the number of confirmed cases of TB.

CONCLUSIONS

Despite advances in the diagnostics and treatment of tuberculosis (TB) this diseases still poses a serious clinical problem. In the studied period the number of positive bacterioscopy results remains stable and observed fluctuations are directly connected with the number of analyzed samples. The number of bacteriologically confirmed positive results of bacterioscopy remains stable so does the number of diagnosed cases of TB in laboratory conditions. In 2009 – 2013, in the Łódzkie Voivodship the number of new diagnosed cases of TB fluctuated. Some cases were noted in children. In 2013 we observed the greatest number of new cases in this age group. However, this observance is not identified with an increase in the number of cases but results from statistical variability. A small percentage of the patients studied for TB demonstrated drug-resistance. However, we noted resistance to basic first-line drugs and occurrence of MDR, pre-XDR and XDR strains.

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