

## Comment

We hand on to the Readers attention the volume consecrated to selected problems of infectious diseases microbiology. Although infectious diseases microbiology belongs to very young medical science domains, has dynamically developed over the last two decades, mainly thanks to application of molecular studies in diagnosis of etiological factors of human and animal diseases. Currently, the classic methods of microorganisms' cultures have been complemented with fast genetic tests confirming the disease process and important microorganism functions. Molecular epidemiology allows to identify the disease's transmissions paths, defining risk factors of this transmission, may characterize interactions between the host and the pathogen, detect microorganism, that cannot be cultivated in vitro and allows better understanding of microorganisms pathogenesis on the molecular level.

Presenting the papers discussing modern studies in certain fields of infectious diseases microbiology to the physicians, we hope, that these will contribute to understanding of many directions of currently conducted scientific studies and the modern diagnostics that we dispose will bring benefit to the healthcare process.

Although we live in XXI century, papers concerning diseases induced by bacteria of *Mycobacterium* species still belong to important healthcare problems among other infectious diseases. Two species – *Mycobacterium leprae* and *Mycobacterium tuberculosis*, two of the oldest bacteria in the world still cause death of millions of humans.

Illustrative article on *Mycobacterium leprae* reminds that the leprosy still remains an important, global health problem, occurring not only in the third-world countries but also in many developed countries including European countries. WHO estimates the number of people affected by leprosy at 10-12 million. Despite its history developing over the centuries, leprosy diagnostics remains difficult, mainly due to many forms and clinical variations of the disease but also because of the lack of in vitro bacteria breeding method. Many phenomenons associated with bacilli survival outside the human body, supposed transmissions between humans, animals and possibly even the environment where bacteria survive, still remain unexplained. Long-term Dapsone monotherapy has led to occurrence of forms resistant to medications and only multidrug treatment recommended by WHO in the eighties began to give desirable outcome.

Acknowledging the *M.leprae* genome in the nineties, provoked the discovery of many unknown bacteria survival features in human environment, and introduction of molecular microbiologic diagnostics to the clinical practice, allowed the identification of specific proteins giving opportunities for immunodiagnostic test development and elaborating new medications and vaccines in the future.

Other disease caused by *Mycobacterium tuberculosis* – tuberculosis – belongs to the group of the biggest human murderers. Number of deaths caused by tuberculosis, are on the second position after HIV and AIDS, concerning approximately 3 million people annually. Although the tuberculosis has been known as a disease for ages, its causing agent has been known for 100 years now and anti-tuberculosis drugs are available, it does not fall in, on the contrary – it increases in many world regions. Important aggravation of tuberculosis epidemic situation has become a fact and this disease cannot be any longer treated as an "ancient disease". WHO recognized tuberculosis at the end of the past century as a population health threat of the global scale. Ethnic issues, war conflicts as well as easy touristic or economic population movements contribute to disease's spread and transmission between humans. Fast patient diagnostics and antibacillary treatment introduction – those two elements belong to the most important methods of fighting tuberculosis. Drug resistant tuberculosis, particularly its MDR-TB and XDR-Tb variants, represent a serious problem of health threat and fight against the diseases on the global scale. Whilst a drug sensitive tuberculosis is a disease completely curable within a standard 6-month period, the treatment of resistant forms often requires 2 years or more and the cure is obtained merely in half of newly diagnosed and in 1/3 of previously treated patients. Also the mortality index is high. Gathering data on the drug resistance tuberculosis prevalence in newly diagnosed (primary drug resistance) and previously treated patients (acquired drug resistance) become an obligation imposed by WHO in the nineties. The paper included in the hereby volume discusses results of 4 consecutive studies conducted according to WHO protocol in Poland.

The serious drawback of drug resistance tests of tuberculosis bacilli, apart from the long result pending period (4 to 5 weeks), is a lack of availability of well standardized methods, which causes difficulties in clinical interpretation of drug resistance phenomenon. Difficulties with tuberculosis bacilli antibiogram standardization constitute only a part of methodical problems concerning also other diagnostic procedures such as: strain cultivation, bacterioscopy and determining the *Mycobacterium* species. Bacilli drug resistance tests are highly specialized test and according to WHO recommendations should be performed in III referral level laboratories. The next article discusses the above mentioned problems and studies results on the evaluation of a new, automatic cultivation system introduced in Poland in order to breed and test drug resistance of tuberculosis bacilli.

As mentioned above, WHO obliged all countries of the world to conduct regular epidemiologic studies covering four main antibacillary drugs. First choice drug group also includes pyrazinamide (PZA), which has not been tested for drug resistance due to lack of standardized method. The work, that we present, is a part of polish studies on the occurrence of drug resistance to PZA of tuberculosis bacilli in polish patients. The studies conclude that this prevalence is unexpectedly high and concerns not solely the strains isolated from the previously treated, but also from newly diagnosed patients. In view of the above fact, PZA drug resistance test should be included to the standard test panel in Poland.

Tuberculosis supervision, apart from early detection of transmitting patients and their treatment, includes also the tracking of infection transmission paths, in the first intention – in individuals from the close patient's surrounding – in order to detect or exclude tuberculosis. Besides, the epidemiologic investigation should cover all individuals who had a shorter or a longer contact with the patient. WHO suggests examining those individuals by assembling them in "epidemiologic circles", determined depending on the time and intimacy of the contact with the patient. Although the exposure of patient's entourage has been known for years, in many countries tuberculosis detection among patient's "contacts" remains still at a low level, which causes an uncontrolled disease spreading. Currently, in epidemiologic investigations of tuberculosis, the molecular methods of bacilli DNA identification are of a great significance. Thanks to these, it is possible to indicate the transmission's direction between two patients with no epidemiologic link. Genetic methods are estimated to increase by 40% the number of detected transmissions, impossible to identify solely by the conventional investigation methods. The article authors in their own studies have confirmed the transmission of drug resistant tuberculosis between the members of examined families and identified particularly dangerous bacilli strains from the molecular family of *Mycobacterium tuberculosis* Beijing 1.

Efficacy and safety of antibacillary treatment may be disturbed in individuals by an easy resistance acquisition by bacilli in contact with a drug, particularly in case of suboptimal doses. One of the factors that may influence the decrease of concentrations of the main antibacillary medication – isoniazid – in the bloodstream is the acetylation process, determined genetically by NAT2 gene mutations. Differences in acetylation dynamics also influence the occurrence of drug adverse reactions, mainly hepatotoxicity, which worsens the treatment regimens. Authors present modern studies on the molecular test detecting the polymorphism of genes inducing the rate of acetylation process in patients treated with INH, indicating its usefulness in tuberculosis treatment monitoring.

Specific feature of *Mycobacterium tuberculosis* is the microorganism transition in latency state (LTBI). WHO estimates the prevalence of this infection on 1/3 of the Globe population. In view of the risk of progression from latent infection to active disease, identification of individuals with a latent infection becomes a key element of tuberculosis prevention programs. Detection of a latent infection with tuberculosis bacilli is also important in other diseases, where it becomes necessary to initiate the treatment with biological medications, which may accelerate the process of transition from latent infection to active tuberculosis process.

Authors present the first comparisons conducted in our country, of two interferon tests IGRA available in Poland, based on protein antigens ESAT-6 and CEP 10, encoded in RD1 region of *M.tuberculosis* genome, absent in the vaccination strain of *M.bovis* BCG. IGRA tests are compared to tuberculin skin test (Mantoux test). They discuss advantages, disadvantages and incompatibility of results of the 3 tests.

Besides humans, tuberculosis is also a disease of many animal species. It occurs in cattle, wild animals, e.g.: aurochs, deers, boars, lynxes, badgers, ferrets and others. In Poland, each year, tuberculosis is detected in animals kept in zoological gardens and in breeding farms. Contagions are promoted by contacts occurring on pasturage and meadows. Tuberculosis transmission may occur in two directions: from animals to humans and inversely – from humans to animals. Thus the early detection of tuberculosis in animals is very important. The next article discusses a post mortem identification of tuberculosis in aurochs, indicating how important a strict supervision of animal diseases is in the supervision of human diseases.

*Mycobacterium* genus, apart from ruthless pathogens, includes also nontuberculous mycobacteria (MOTT – mycobacteria other than tuberculosis, NTB – nontuberculous mycobacteria), which may be a cause of clinically and radiologically undistinguishable diseases in human – mycobacterioses. At the same time their natural habitat is the human environment – mainly soil and water. When they become a cause of disease, they require a treatment, often longer than in case of tuberculosis and with a use of multiple drugs. Therefore molecular works on the most frequently occurring bacilli species – etiological factors of mycobacterioses belong to the group of modern studies. Authors present *Mycobacterium kansasii* genotyping, MOTT bacilli species most frequently occurring in Poland both in patients suffering from pulmonary silicosis, COPD, cystic fibrosis, cancerous diseases, AIDS and HIV infected individuals as well as in the environment.

Studies on *Bacillus anthracis* causing the infectious disease called anthrax have lasted for 100 years now, i.e. from the moment of its' discovery by Robert Koch. Although herbivore animals are mainly susceptible to infection, the diseases may occur in omnivores, carnivores and humans. In human, depending on the contagion way, anthrax may occur in three forms: skin form, associated with a skin lesion and dermatitis; pulmonary form, as a result of inhalation of spores or intestinal, as a result of consuming contaminated food or water. Expediency of

anthrax studies is argued by the possibility of the import of contaminated supplies from the countries, including those neighbouring Poland, where anthrax still occurs enzootically as well as its main role as biological weapon factor in bioterrorist acts. Authors employed molecular methods do detect *B.anthraxis* and to distinguish it from other non-pathogenic microorganisms occurring in the environment: *B.cereus*, *B.mycooides* and *B.thuringiensis*.

Besides many features of virulence, resistance to antibiotics, the other feature facilitating the microorganisms' survival in the stage of invasion is the ability to form a biofilm. Many species of microorganisms possess this ability. Biofilm is a structured bacterial cell population attached to the surface, demonstrating different phenotypic features from cells occurring in the planktonic form such as: decreased sensitivity to drugs, increased invasiveness and others, which contributes to prolonging the inflammatory state and impeding the healing process. Furthermore, different bacteria species creating biofilm may co-exist with each other intensifying the problem. The next article discusses the ability of *Staphylococcus aureus* strains isolated from patients to create a biofilm, along with its' genotypic analysis. The article is preceded with an extensive overview of biofilm formation and its' negative role in infection chemotherapy.

The next article discusses difficulties in clinical and microbiological diagnostics of pleural exudation etiology in humans. When it comes to infectious causes, in Poland we lack reliable data on the prevalence of different diseases with pleural exudations. Authors, basing on the diagnostic of over 2500 patients treated in surgical and medical departments, presenting pleural exudation, analyze bacterial cultures results and determine the bacterial drug resistance patterns in association with proposed clinical diagnosis.

In nosocomial infections, to which patients undergoing medical procedures are exposed, different fungi species constitute an important factor. *Candida parapsilosis*, which is discussed by the authors of the next article, is an important factor of candidial, catheter induced placentitis. Its virulence is intensified by the ability of colonization of artificial surfaces and formation of biofilm. Furthermore, the species infecting human, occurs in many phenotypic forms, difficult for microbiological identification, impeding yet complicated fungi diagnostics. Authors observed the occurrence of phenotypic variability in approximately 20% of cultures derived from patients. *Candida parapsilosis* occurred in 4 main morphotypes with biochemical features variability and various abilities to form a biofilm. These studies have an important significance for fungal infections diagnostics in patients.

Among numerous fungal infections in the modern world, skin and skin derivatives remains its' most frequent localization. Many fungal species participate in this process: dermatophytes, candida-like fungi and mold. Although in most cases surface mycoses do not threaten the patients' life, in many cases they are burdensome and transmit between humans. For individuals with immunosuppression they may constitute a portal of entry for systemic mycoses caused by mold fungi. Despite the continuous progress in diagnostic and treatment of superficial fungal infections, they still constitute a serious therapeutic and social problem, and due to the possibility of infecting other individuals, also an epidemiologic problem. Authors analyze in their article, fungi species isolated from over 2000 patients referred by doctors of different specialties with a suspicion of superficial mycosis. Authors discuss microbiological methodology, anamnesis conducted with patients before examination and analyze species isolated from different parts of the body responsible for infections. Presented data may be useful in empirical drug prescribing, in situations where there is no possibility to perform a full microbiologic examination. However it should be always aimed to perform such diagnostics.

#### Conclusion

The goal of microbiologic diagnostic is the search and identification of the pathogen. Accurate diagnosis relies on sample drawing, the method of its' transport and/or storing as well as the results interpretation. The result itself, without a proper bacteriological interpretation does not fulfill its' role and may be misunderstood by the physician.

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