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Dear readers,

Starting with the fourth quarter of the year 2015, we rejoice to announce you the release of the first issue of the annual publication in English of *"One Health International Journal",* which will replace the "International Journal of Comparative Oncology", under the auspices of both "One Health – New Medical Concept" Association in Romania and the Mediterranean Forum of Comparative Oncology.

We invite you to submit articles relating to all the component elements of *One Health* concept, to be found in the hexagon on the cover. This is a brand new domain in the international medical world, and, therefore, the desideratum is also valid for Romania. Under these circumstances, it is not possible for our journal to benefit, at the present moment, as would be normal, from the expertise of a small group of *One Health* senior specialists, who would gather to take part in a "little reunion".

This little reunion would be convened by the board members, who, in our case, are the most representative specialists in this super new domain, and who are currently completing their spectrum of multidisciplinarity and multifactoriality. Please note, as a peculiarity, that the board members of the journal, which will be released bi-annually (in May and November), will submit scientific articles for publication as well.

Some of these super specialists have an experience of over 50 years in the field.

The board members are, at the same time, the very professors of future "young specialists", who, in their turn, will submit future articles to be published in our journal.

The Journal Structure (the articles that will be published in the second issue of the journal) will be similar to the conceptual hexagon sides, namely they will contain facts and events from:

- The Comparative Medicine, that brings together both human and veterinary medicine
- The Environmental Medicine, that will comprise elements referring to the existence, monitoring and elimination of potentially biotic factors in air, water, soil and plants, thus establishing "the primary prophylaxis for human and animal diseases"
- The Occupational Medicine, that will encompass both sociological and family-related elements, which is an extremely diversified domain
- Zoonoses - along their entire epidemiologic chain, with a special emphasis on borderline forest zoonotic and vector-transmitted diseases – responsible for maintaining these pathologies
- The Comparative Oncology – another sector, the oldest of the hexagon (over half a century old), that will make a major contribution to the newest domain, namely cancer immunotherapy for humans and animals
- The Food and Nutrition Medicine, that, through its interdependent reunion, will create the optimum premise for healthy food and healthy nutrition notions

All the above mentioned components will constitute, through the specific articles submitted for publication, the most convincing propagandistic elements for the identifying, sustaining and promoting the new *One Health* concept.

Editor in chief,

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Altruism and humanist One Health

Bucharest June 2016

In full of European rationality the hipocratic vow looks increasingly obsolescent. Slightly discordant with the times this act of commitment appeals to a set of ethical rules, the rules that configure the Crown of professional morality. Practitioners and their disciples invoke the sacred connection with "*the pain*" with teachings gleaned as a debt of gratitude which picks up on the fly and willingly. Thus, the practice of medicine cannot be reduced to a profession but is built to a vocation. Otherwise you're considered "*traitor and perjury*".

The value of the professional consists in prudence, patience, education and medical scientific skills.

Analysis of philosophical ethical text shows a parallelism between the personal and the professional life of the doctor in his relationship with the human or animals species….. *"clean and sacred I will maintain my life and my art*…." .

How current over the centuries, are the precepts and fundamental principles of medical practice regarding observance of professional secrecy, the autonomy of the patient, the physical and moral protection of the life, *"….drive away any suspicion of corruption…."*.

For the first time the idea of advancing prevention Hippocrates, the influence of climate, food and thinking in the wellbeing of the body and treat suffering into a unified whole. Any lack of poise between these elements can lead to suffering.

Theoretically, the rules are the basis of the concept of the innovative philosophical concept of primary prevention One Health.

Our development as human beings within the ecosystem in harmony with the world species depends on the availability of each to share emotions, fears, doubts, opinions, unrest, rationalizations, justifications in relation to a supreme judge. To show this availability should we train. How much education is deeper with both the power of understanding, sensitivity are higher.

Harmony between public health, the ecosystems in which we evolve, the epidemic control of which benefit, and human spirituality are the aim of professional conscience.

The arrogance of the man to subordinate spiritually, the will of the people, the good faith generated social inequality and non-away from the philosophy and humanism of hippocrates oath.

More than ever there is a need to return to the symbiotic connection between flesh and spirit, to recover professional ethics in favor of patient.

Unfortunately, beyond the personal and professional sacrifices are sometimes subjected to twenty-first century, doctors often ignored by the officiels, with an embarrassing social status, are forced to a survival politics.

In a society without moral reference points, lacking confidence, dominated by day care tomorrow, consumers of the moment confuse freedom with debauch.

Handled by the older of the day they prefer the superficiality, the ephemeral satisfaction of the moment.

Leaving the principles has led to the loss of the humanist altruists having been replaced by the survival solution.

Dr. Florian Udrea

EXPLANATORY NOTE

The articles in the current issue of "One Health - International Journal" (2/2016) do not conform to the classic presentation of a scientific paper submitted for publication because they are informative materials that were presented at a scientific session organized by "Dr. Victor Babes" Hospital for Infectious and Tropical Diseases in Bucharest - Romania this year. The importance of these pieces of information and the urgent need to be immediately disseminated did not allow the respective texts to be reformulated under the traditional publishing conventions.

SUMMARY

ZOONOSES – COMMON INFECTIOUS DISEASES IN HUMANS AND ANIMALS

N. Manolescu, E. Ceausu
The Romanian Academy of Medical Sciences

The World Health Organizations defines zoonosis as „Any disease or infection transmissible through natural pathways from vertebrate animals to humans or from humans to vertebrate animals."

Zoonoses, common infectious diseases between humans and animals, are produced by an impressive and extremely varied number of pathogen agents (prions, viruses, bacterium, fungi and parasites). So far there have been identified over 1400 pathogenic agents which produce diseases in humans, 800 of those represented by zoonoses.

It is believed that at least one third of infectious human illness is of zoonotic origin.

In the past 30 years there have been identified 180 pathogenic agents which have produced new, emergent or reemerging human infectious diseases. Out of these newly identified agents, 130 come from animals, most of which are RNA viruses.

Some zoonoses are asymptomatic or manifest through an unspecific clinical tableau (flu like symptoms, benign manifestations, etc.). Others have severe manifestations (hemorrhagic fevers, rabies, plague, Hantavirus infection, arboviral infections, SARS, etc.) or leave important side effects (Lyme disease), or/and present an important epidemiological potential (avian flu, swine flu, SARS, etc.).

Zoonoses are transmitted to humans directly or through insect vectors (mosquitoes, etc.). They can manifest sporadically, endemically or pandemically (avian flu, SARS) in both humans and animals. Some zoonoses manifest identically in both humans and animals, while others present with different manifestations.

Their presence and incidence in humans depends on their presence and incidence in the territorial animal population, both domestic and wild. In developed countries, including Romania, an important animal population is also represented by pets. In the USA, there are over 200 million pets, out of which over 100 million are represented by cats and dogs, which enable the transmission of over 250 different pathogenic agents and over 100 million cases of animal bites a year with a high risk of human infection.

In Romania, there are a large number of stray dogs and cats, as well as a large (unknown) number of household pets. Dogs can transmit over 60 diseases to humans, and cats over 40 diseases (rabies, Q fever, leptospirosis, toxoplasmosis, echinococcal disease, ascariosis, etc.)

Zoonoses cannot be eradicated because of the existence of a large animal population in nature. They can spread easily between territories, between countries, from one continent to another through the natural trans-border migration patterns of wild animals (mammals and birds), through the transport of domestic animals and pets, through meat food products and vectors (mosquitoes, fleas, ticks and other insects). Because of these factors, epidemic outbreaks are unpredictable and hard to control.

The last century has brought major changes in the industrial use of land, changes in agricultural production including the raising of farm animals, the destruction of natural habitats (forests, lakes, swamps, and mountain grazing areas), the rise in human activity and human transcontinental travel, and an increase in circulation of animals and animal food products. All of these factors have contributed to a

change in the distribution, presence and density of zoonoses hosts and vectors, as well as zoonotic pathogen agents. As a result, the number of emergent and reemerging diseases, that also include zoonoses, is in continuous rising (West Nile virus epidemic in Romania in 1996 and in the USA in 2000, SARS in 2003, avian flu in 2004, MERS-CoV in 2012, Ebola in 2014). It is believed that almost 60% of recent emergent infectious diseases come from animals, and 75% of these specifically originate from wild animals.

As well as the modern human's traveling habits, we should also take into account the migration patterns of animals between their natural habitats, as well as the more common interaction between wild animals and both domestic animals and humans.

Zoonoses represent an important preoccupation of human and veterinary medical services in every European country, because of the high number of cases presenting in humans as well as the possibility of epidemical outbreaks. According to the European Center of Disease Prevention and Control (Animal Epidemiological Report, 2014), the most important zoonoses confirmed in humans in 2012, taking into account number of cases, seriousness of disease and epidemiological potential, have been: Campylobacteriosis with 215.217 cases (92 in Romania), Salmonellosis with 92.911 cases (755), verotoxin Escherichia Coli with 594 cases (1), Listeriosis 1.642 cases, Q Fever with 649 cases (16), Brucellosis with 403 cases (0), Trichinellosis with 378 cases (220), West Nile fever with 232 cases (15), congenital Toxoplasmosis with 40 cases (0), Botulism with 102 cases (19), Cryptosporidiosis with 9.591 cases (0), Echinococcal disease with 818 cases (98), Leptospirosis with 799 cases (77), Denga fever with 1.177 cases (3), Anthrax with 20 cases (14), tick borne encephalitis with 2.553 cases (3), etc. The real number of cases is in reality larger (unreported or undiagnosed cases, etc.) in a lot of EU countries, including Romania. In Romania, some zoonoses like Listeriosis, Cryptosporidiosis, Campylobacteriosis, Hantavirus disease, tick encephalitis, etc. are under diagnosed from both a clinical point of view (through lack of medical information) as well as a laboratory investigations (lack of specific laboratory equipment). In Europe, Romania is one of the leading territories in zoonostic infections (Trichinellosis, Leptospirosis, Anthrax, Botulism). Visceral Leishmaniasis, currently an imported zoonotic disease in Romania from the Mediterranean regions, will in future years become endemic in our territories because of increased global warming and vector migration to the North of the Danube River. The last years have seen an important increase in the number of human Borreliosis cases and, in veterinary clinics, an increase in the number of Bartonellosis cases in pets, with secondary infections in humans that mostly remain undiagnosed.

According to the guidelines put forward by the Ministry of Health in 2015, out of the 65 diseases with the biggest impact on public health, 30 of them are zoonoses because of their high transmission potential at a national and international level and the severity these diseases represent.

The surveillance and control of zoonoses can only be achieved through cooperation between human and veterinary medical services. The information and/or accomplishments in human medicine or in veterinary medicine generate information and accomplishments in the other.

Human and veterinary doctors play an important part along with epidemiologists and ecologists in the study of the causes zoonotic epidemics/pandemics and in the minimalization of the effects of these diseases. Human and veterinary doctors represent the first line of defense in the detection of a zoonosis or zoonotic epidemics because they represent the first interaction of patients with specific clinical manifestation and of diagnosis of zoonotic diseases in animals or in the wild.

The transmission of zoonoses from animals to humans is done through respiratory pathways (Flugge drops from the animals breathing, the inhalation of dust contaminated from saliva, fecal matters, urine), through direct tegumentary and mucosal contact, through scratching, biting, through oral pathways (unwashed hands after contact with an animal or animal byproducts, the ingestion of contaminated fruits or vegetables), through contact with contaminated objects (cages, leashes, etc.), and through vectors (fleas, mosquitoes, flies, etc.).

In humans, zoonoses manifest in extremely varied ways, with symptoms that can be respiratory, of the central nervous system, skin manifestations, etc. Some diseases can have severe clinical manifestations and have a high fatality rate.

Human Disease	Fatality Rate %
Creutzfeldt-Jakob Disease	100
Rabies	100
Anthrax - Inhalatory	80 - 90
Simian Herpes	50 - 75
Ebola	70
Easter Equine Encephalitis	50 – 70
Hantavirus Pulmonary Syndrome	60
H5N1 Flu	15 – 50
Yellow Fever	20 – 50
Nipah Virus Encephalitis	50
Lassa Fever	15 – 52
Plague	50 – 80
Rocky Mountain Spotted Fever	20-60
Skin Anthrax	20
Tularemia Pneumonia	30-60
Visceral Leishmaniosis	5-25

BACTERIAL ANTIBIORESISTANCE: MEDICAL, SOCIAL AND ECONOMIC ASPECTS "PRESENT AND FUTURE"

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Bacteria have been present on Earth since 3.5-3.8 billion years ago, while humans for only 200,000 years. Over 50% of the Earth's biomass is represented by bacteria, out of which only 5% have been classified. 1 gram of soil contains between tens of thousands and several million bacterial species**.**

Everyone on the planet harbors billions of bacteria (1.5 Kg of which up to 1 kg in the digestive tract). 1 g of saliva contains 1 billion bacteria and 1 g of faeces 100 billion bacteria. Bacterial resistance to antibiotics is a natural phenomenon appeared long before humans have discovered how to use antibiotics in medical practice.

A lot of antibiotics are produced by bacteria (e.g. Streptomycin produced by *Streptomyces griseus*). The reason for secretion of bacterial antibiotics is the elimination of competing bacteria from the environment.

Antibiotic-producing bacteria also develop enzymes that simultaneously protect them from substances with antibacterial effect.

By transfer between bacteria, the genes responsible for coding these resistance enzymes are transmitted to other species, and these in turn become resistant to antibiotics.

This natural process has increased enormously in recent decades due to:

a) The use of antibiotics in human and veterinary medicine (treatment, prevention), in animal breeding and aquaculture (growth factor), in agriculture (treatment of bacterial diseases of fruit trees, flowers, vegetables), and in industry (industrial paints).

b) The massive use of industrial and residential biocides chemically similar or even identical to some antibiotics.

c) The use of disinfectants in hospitals and at home.

d) The intensification of the movement of resistant bacteria strains in nature, animals and humans and between animals and humans**.**

Over 50% of the antibiotics produced in the world are intended for veterinary use (approximately 27,000 tons). In 2011, over 17,100 tons antibiotics were sold in the US, out of which 3,500 tons for human use (20.5%) and 13,600 tons of veterinary use(79.5%)**.**

In 2007, 3,350 tons of antibiotics for human use were used in 29 different countries in Europe. World consumption of antibiotics has increased by almost 40% between the years 2000 and 2010. 40% of antibiotic prescriptions given by doctors are not required (WHO). Antibiotic resistance is now a serious threat to public health,its presence and growth due to both usual antibiotics and reserve antibiotics.The world is heading towards a post antibiotic era, where current infections may kill us again(WHO 2014).

UK Prime Minister David Cameron has said that the rapid development of world wide bacterial resistance is a threat and that "if we fail to act, we are looking at an almost unthinkable scenario where antibiotics no longer work and we are cast back into the dark ages of medicine where treatable infections and injuries will kill once again." (2014)

Bacteria resistant to antibiotics currently cause at least 700,000 deaths / year world wide including 27,000 in Europe and 23,000 in the US (2014)**.**

It is believed that by 2050 further increase of bacterial resistance to antibiotics will cause up to 300 million deaths (10 million deaths / year), causing a decrease of global GDP by 2-3.5%. Of the 10 million deaths / year, most will occur in Asia (4.7 million) and Africa (4.1 million) and the fewest in Europe (390,000) and United States (317,000).

By 2050, death by infections from resistant bacteria will be the first cause of death in the world, ahead of cancer (8.2 million deaths / year), diabetes (1.5 million), diarrheal disease (1.4 million) or road accidents (1.2 million) (UK Report, 2014).

In the next 35 years, the economic cost of infections caused by antibiotic resistant bacteria will be up to 60-100 trillion. In the US overall cost of hospital infections caused by antibiotic-resistant bacteria is already over $ 20 billion / year.

Consumption of antibiotics in Europe in 2012 (ESAC-Net) averaged 21.5 DDD / 1000 inhabitants / day; the lowest consumption was in Northern Europe (Scandinavia and the Baltic States), the highest consumption in Greece and Romania (> 19.6 DDD / 1000 inhabitants / day). Consumption of cephalosporins type III in EU averaged 1.7- 1.9 DDD / 1000 inhabitants / day compared to Romania where it averaged <5.2 DDD / 1000 inhabitants / day (overall consumption in the community and the hospital).EU consumption of ciprofloxacin in 2012: 0.35 DDD / 1000 inhabitants / day in U.K., 1.7 DDD / 1000 inhabitants / day in Luxembourg and <3.5 DDD / 1000 inhabitants / day in Romania.

According EARS Net in 2013: there were reported 5.0%*E. coli* ESBL-producing isolated from systemic infections in Iceland and 93.6% in Bulgaria. Romania reported *E. coli* ESBL + in 10-25% of cases.In 2013, *Klebsiella pneumoniae* producing ESBL strains isolated from systemic infections were reported in 0% of cases in Iceland, 70.1% in Greece. Romania reported more than 50% strains of *Klebsiella pneumoniae* ESBL +Carbapenem resistant strains, and the reports of *Kb. pneumoniae* reported in 2013 in the EU averaged 8.3% as follows: 0% in Bulgaria, Finland, Iceland, Sweden etc. and 59% in Greece. Romania reported 10 to 25% of invasive strains of *Kb. pneumoniae* resistant to carbapenems. Resistance to penicillin G for strains of *Str. pneumoniae* in 2013 were as follows: 1.1% in the Netherlands compared to 40.0% in Cyprus; resistance to macrolides 1.5% in Lithuania compared to 38.1% in Romania. Reports about MRSA Staphylococcus aureus isolates in 2010-2013 were: 0% in Iceland compared to 64.5% in Romania.

In the last 10 years very few new antibiotics have been introduced in therapy because international laboratories have virtually abandoned research on antibiotics, mainly due to economical reasons. We are currently witnessing a growing imbalance between the numbers of new antibiotics for clinical use and rapid growth of emergence of bacterial resistance, which makes the risk of therapeutic impasse more frequent. To cope with this situation, it is not important to find a solution that can prevent the emergence of resistance, because bacteria will always find a way to adapt, but rather preserve the effectiveness of antibiotics available for a longer time.

THE DYNAMICS OF BACTERIAL ANTIBIOTIC RESISTANCE IN "DR. BABES" HOSPITAL FOR INFECTIOUS AND TROPICAL DISEASES(2000- 2015)

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Keywords: RealTimePCR, carbapenemases, KPC, metallo-beta-lactamase NDM, OXA48.

Introduction:

Antibioresistance is not just acyclical or transitorial phenomenon, but an intrinsic property of the bacterial world. The variability of resistanceis linked to the occurrence of mutations in bacteria or their ability to accept foreign DNA.

The future of antibiotics should be judged on the basis of the research results and their use in medical practice in the past decade.

Starting with the1950, "the golden era" of antibiotics begun. Then was the time when chemical synthesis of beta–lactams and quinolones started and research increased exponentially.Even with the very encouraging obtained results, some scholars thought it unwise to declare that the problem for treating infectious diseases has been solved.

Giving broad spectrum antibiotics to people has severe consequences. Antibiotics kill not only microbes involved in an infection but also commensal microbiota, leading to a full body unequilibrium (dismicrobism)that allows opportunistic bacteria to colonize and trigger pathogenic mechanisms with serious clinical manifestations.(Example: ulcero-necrotic diahreea produced by Clostridium Difficile infection, post antibiotic micotic vaginitis produced by Candida Albicans). There is also a natural selection of resistant strains in the microbiota of the body even when bacterial dismicrobism is not installed. Microbiota pathogens of the human body can themselves cause hospital acquired infections.

Vancomycin treatment against methicillin resistant *Staphylococcus aureus* infectious (MRSA) or against acute diarrhea with *Clostridium difficile,* has led to colonization and urinary infectious with vanco-resistant *Enterococcus faecium* (VRE).

Bacteria have the ability to develop resistance to antibiotics through a variety of mechanisms, whose nature and efficiency depend on the species, but also through the chemical characteristics of the used antibiotic.Bacteria also have tremendous ability to mutate and to exchange genetic material.

In US it is estimated that about 50% of antibiotics are administered incorrectly, frequently in viral infections, and often in improper doses or dosing intervals. In recent years, antibiotics were administered excessively worldwide, considering that only a few new molecules of antibacterial agents have been discovered.

One of the biggest sources of antibiotic resistance is antibiotic treatment to animals in farms and embedding antibiotic in food so that they can grow and be slaughtered quickly.Urban congestion, increasing number of homelessness, poor nutrition and poor hygiene, lack of proper medical care, all these can lead to the spread of antibiotic resistance, both in economically developed countries but especially in developing countries.

In these countries, the social organization of health care and hospital networks represents a source for the acquiring resistance genes from bacteria, which then spread through the visitors and caregivers to the community.Tourism growth and international travel is another way for spreading worldwide multi-resistant bacteria.

Other sources of antibiotic resistance are the immunosuppressed patients, AIDS patients, invasive medical procedures, organ transplantation, medical device implants (which involve the formation of biofilm) etc. All these factors are involved in the increased incidence of bacterial infections both in the

community and in multi-drug resistant (MDR)hospital acquired strains.It is important to emphasize that bacteria can combine several types of resistance mechanisms to strengthen their defensive shield against the action of antibiotics.

The techniques of antibiotic susceptibility testing are a critical goal for microbiology laboratories.In the era in which antibiotic resistance has become a common phenomenon and there is an emerging resistance to multiple antibiotics (especially in Gram-negative bacteria where only a few therapeutic options have remained), the importance of a quick and brief report about the sensitivity of an isolated strain must not be under estimated.The results of an antimicrobial susceptibility test are not only a guide for individual patient management, but the sum of results that may be included in a guide about empiric therapy.The first step in preparing the report on antibiotic resistance is the development of protocols that specify which microorganism isolated from a biological sample is significantly involved in the infectious process and after which the antibiotic sensitivity test should be performed.

This decision is made in the clinical context and depends on several factors: age, sex, clinical specimen type, history of recent illness, recent hospitalizations (in past year), multiple hospitalizations (especially in the ICU), pretreatment with antibiotics etc.

It should be noted that some bacterial species show intrinsic resistance to certain antibiotics,while others show predictable patterns of sensitivity to certain classes of antibiotics (ex: penicillin-susceptible *Streptococcus pyogenes*) and, according to the CLSI Standard, they no longer have to be tested.

The time required to obtain the first results for antibiotic sensitivity tests depends on the required time frame for the etiological agent to grow in pure cultures, i.e. 6-48 hours. To this, there needs to be taken into account the antibiotic susceptibility testing, and if the particular strain shows resistance phenotypes (ESBL carbapenemases, resistance to vancomycinetc), further tests are needed.In automatic techniques (MicroScan, VITEK, etc.) sensitivity testing results could be obtained in 6 to 8 hours from the isolation of bacteria in pure culture.

1. β-lactams and *Staphylococcus spp-* interpretative sensitivity

Staphylococcus is a Gram-positive bacterium with a natural sensitivity to β- lactams (except monobactams).They have a great capability to quickly adapt to the pressure caused by antibiotic treatment and can acquire resistance.Ceftobiprol and ceftaroline have affinity for PBP2a and they are clinical efficient against methicillin resistant strains. (8.9).

For *Staphylococcus aureus*, resistance to fluoroquinolone also suggests methicillin resistance. Methicillin-resistant strains of *Staphylococcus aureus* acquired in community infections (CA-MRSA) are sensitive to most classes of tested antibiotics, including fluoroquinolones.

***2. Enterobacteriaceae* sensibility to β- lactams**

Inactivating enzyme production is the most important mechanism of resistance to β- lactam antibiotics, in the *Enterobacteriaceae*. The great diversity of existing β-lactamases is divided into two major groups based on structural and functional criteria:

1. Ambler structural classification (1) based on amino acids sequence from the conserved elements of the active site and

2. Bush and G.A Jacoby Functional Classification (4) based on hydrolytic activity and β-lactamases sensibility to inhibitors likeclavulanic acid and EDTA (divalent cation chelator)

Resistance phenotype of strains producing extended-spectrum of β- lactamases (ESBL)

The resistance phenotype characteristic ofextended-spectrum β-lactamases (ESBL) producing *Enterobacteriaceae* strains is characterized by resistance to penicillin and cephalosporin, with exception of cephamycin.

Carbapenems and cephamycins are not usually a substrate for these enzymes. ESBL production is due to multiple therapeutic failures. Except for β-lactamase inhibitor combinations, cephamycins, carbapenems and β-lactams have to be reported as "intermediaries", rather than "sensitive" if the synergy test is positive.

ESBL were described in Western Europe in the 1980s, when C3G were widely used in ICUs.Today they are present in both the community and hospital acquired infections. The prevalence is almost identical in France (1-3%) and Germany (1-5%), higher in Italy (9-15%), Great Britain (7-22%), Eastern Europe (Russia, Poland, Turkey, Greece): 39-47%.

Cephalosporinases overproduction ("High-level cephalosporin")

The resistance phenotype determined by high-level synthesis of cephalosporinases is characterized by marked resistance to penicillin, C1G, at least one of C2G, C3G or aztreonam. Synergism

test is negative between C3G, C4G or aztreonam and β-lactamase inhibitors. Cephamycins are inactive. Instead, C4G remain active.

The prevalence of resistance phenotype through high cephalosporinases production among the species of the *Enterobacteriaceae* family isolates from nosocomial infections in Europe is about 5-40%. This phenotype is characteristic of *Enterobacter cloacae* species, *Enterobacter aerogenes*, *Serratia marcescens*, *Citrobacter freundii* and sometimes *E. coli*.

Carbapenems resistance

Carbapenem resistance in the *Enterobacteriaceae* family is determined, in particular, for one of the 3 carbapenemases classes: Class A Ambler (*Klebsiella pneumoniae* - carbapenems / KPC), Class B (metallo- beta lactamaze- MBL / Verona integron- encoded matallo - beta-lactams (VIM), New Delhi matallo-beta-lactams (NDM) and class D (oxacilinaze / OXA-48) in combination with extended spectrum producing beta-lactamases / ESBL.

The carbapenemases class represented by KPC ("*Klebsiella pneumoniae* carbapenems") was first discovered in North Carolina in 1996 and then spread quickly through Europe, South America, Middle East, and Asia. They confer resistance to all β-lactams, including cephamycins, C3G, C4G, carbapenems. Overproduction of cephalosporinases associated with the waterproof membrane (porins alteration) is a common cause of resistance to carbapenems in *Enterobacteriaceae*. This phenotype of combined resistance is particularly observed in strains producing chromosomal cephalosporinases (*Enterobacter aerogenes*, *Enterobacter cloacae*, *Citrobacter freundii*).

3. *Pseudomonas aeruginosa* sensitivity to β- lactams

Pseudomonas aeruginosa naturally produces small amounts of cephalosporinases (AmpC), which contribute to the intrinsic resistance in this species and the natural expression of the four efflux systems, in which MexAB-OprM is the most important. It is resistant to several β-lactams with hydrophobic properties (waterproof) such as benzylpenicillin, oxacillin, aminopenicillin, C1G, C2G (cephalothin, cefuroxime), C3G (cefotaxime),moxalactam, carbapenems. Wild strains of *Pseudomonas aeruginosa* are sensitive to carboxypenicilline (ticarcillina, carbenicillina) ureidopenicilline (piperacillin), some cephalosporins (cefsulodin, ceftazidime) at monobactams (aztreonam), carbapenems (imipenem, meropenem, doripenem).

In Europe, according to the latest study MYSTIC 2006, imipenem resistance was around 32%, 25% to ceftazidime, 22% for meropenem, 15% for piperacillin- tazobactam. Other European data (EARSS 2007) shows a difference of resistance between Northern European countries and Switzerland compared to antibiotic resistance in Southern Europe. For example, resistance to imipenem ranges from 5.4% in Switzerland, to 32.5% in Italy.

4. *Acinetobacter baumannii* sensitivity to β- lactams

Acinetobacter baumannii, a coccobacillus shaped Gram-negative bacterial species, is one of the emerging bacteria which poses a serious challenge in medical intensive care units. It affects immunocompromised patients of various causes, patients of extreme ages, with serious illness, or under assisted ventilation.

MDR *Acinetobacter baumannii* epidemic strains are known to be resistant, usually to all β-lactams, fluoroquinolones (chromosomal mechanism) aminoglycosides (inactivating enzymes 16S-rRNA or for methylases), and they remain susceptible only to colistin, tigecycline, rifampicin. There is a further resistance to the latter antibiotics that leads to lack of treatment options.

Objectives:

Analyzing the dynamics of global antibiotic resistance of some bacterial species isolated from patients admitted to the "Dr. V. Babes" Hospital for Infectious and Tropical Diseases compared to the same antibiotic resistance in bacterial species isolated from patients in ambulatory care (FVB), in 2000-2015.

Material and Methods:

Antibiotic resistance profiles of bacteria isolated from patients admitted to the "Dr. V. Babes" Hospital for Infectious and Tropical Diseases between the years 2000 to 2015 were identified by the standard diffusion method and MIC values by E-test methods and VITEK2C.

Reading of results and compliance with sensitive categories / S, intermediate / resistant I / R as well as the recognition associated mechanisms of resistance was performed according to standard criteria of CLSI and EUCAST.

Screening of ESBL-producing isolates was performed by the double disc diffusion method, which highlights the synergy between beta-lactam beta-lactamase inhibitors and / clavulanic acid, sulbactam.

Cephalosporinases AmpC production was highlighted by E-test method using strips impregnated with cefotetan /cefotetan + cloxacillin. Bacterial isolates producing carbapenemases were identified by determining the MIC to meropenem and imipenem through E-test, using cards VITEK / AST084 and AST222, phenotypic Hodge test, modified and bands E-test impregnated imipenem/imipenem + EDTA (IP / IPI / bioMerieux), chromogenic medium for screening ESBL producing strains and carbapenemases (ESBL and ChromCarba- Chrom R / bioMerieux), rapid screening test OXA-48 (immunoassay), RealTime PCR / GeneXpert. Extraction of DNA from cultures of Klebsiella pneumoniae was performed by "MasterPure ™ Complete DNA and RNA Purification Kit" (Epicentre, Biotechnologies, Madison, Wisconsin), and detection and quantification of bacterial DNA with The Primer Design ™ Kit (Primer Design 2X Precision ™ MASTERMIX) and 32 LightScanner Tools / LS32 (Idaho Technology, Salt Lake City, UT).

Internal quality control: Staphylococcus aureus ATCC29213, ATCC49619 Streptococcus pneumoniae, E. coli ATCC25922, Pseudomonas aeruginosa ATCC27853.

Results:

Testing, knowledge and analysis of multi-annual dynamics of local antibiotic resistance, is an obligation of microbiologists due to the incredibly useful information they provide to fellow clinicians, in order to choose the most effective therapeutic decisions.

1. ***Staphylococcus spp*. methicillin-resistant**: dynamic analysis of the incidence of *Staphylococcus aureus* MRSA in "Dr. V. Babes" Hospital for Infectious and Tropical Diseases in the period 2000 to 2015,highlights an increase from 12.2% (2002) to 42.7% (2013), 40.4% (2015). Strains of *S. aureus* / MRSA isolates were collected from different body sites: secretions collected from skin lesion superficial and deep, respiratory infections and ENT, systemic infection, etc.

Fig. 1:

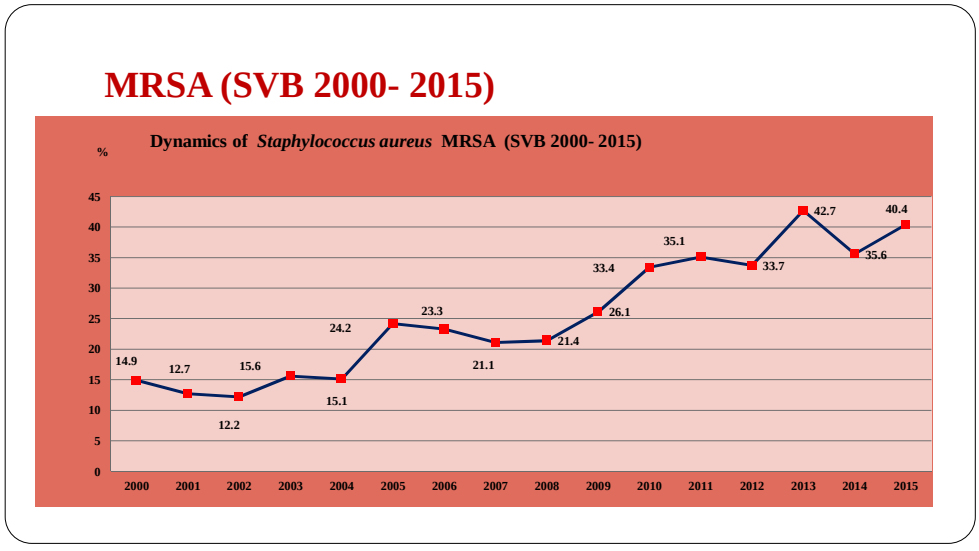
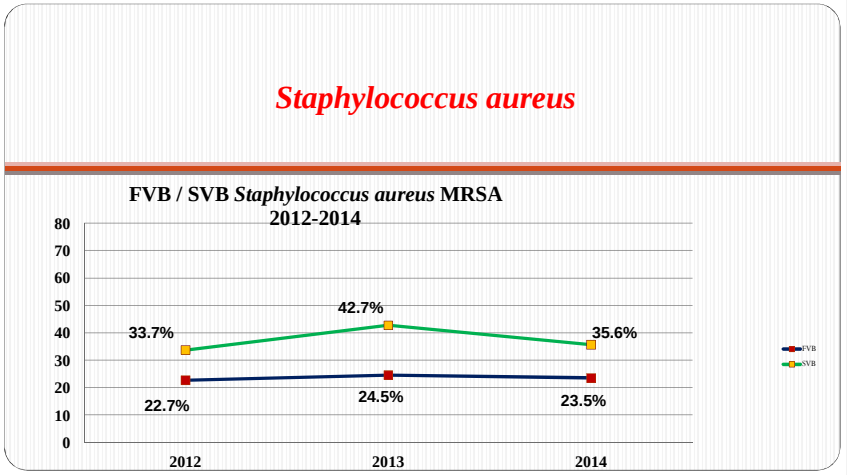


Fig. 2: The dynamics of MRSA isolation in hospitalized patients (SVB) versus outpatients (FVB)



In 2012, 33.7% MRSA strains were isolated from hospitalized patients (SVB), compared to 22.7% of MRSA strains which were isolated from ambulatory (FVB). A percentage of 42.7% of MRSA strains was registered in 2013 in SVB, along with a slight increase of 24.5% in outpatients (FVB).

2. **The dynamics of macrolide resistance strains of *Streptococcus pyogenes* - SVB 2000-2015:** In the past 3 years, SVB microbiology lab found a sharp increase in the incidence of erythromycin resistant strains of *Streptococcus pyogenes*, due to administration of macrolides in respiratory infections, in ENT and skin infection. This therapeutics approach is common in primary care or in cases of self-medication. In 2010 we recorded a 3.2% erythromycin resistance, and in 2011, a sharp increase from 10.1%, that has been maintaining over 10% in the following years. In 2015 we registered 20.3% of macrolide resistant *Streptococcus pyogenes* strains isolated from patients admitted to SVB and 28% in isolates from patients from ambulatory care (FVB).

Fig. 3:

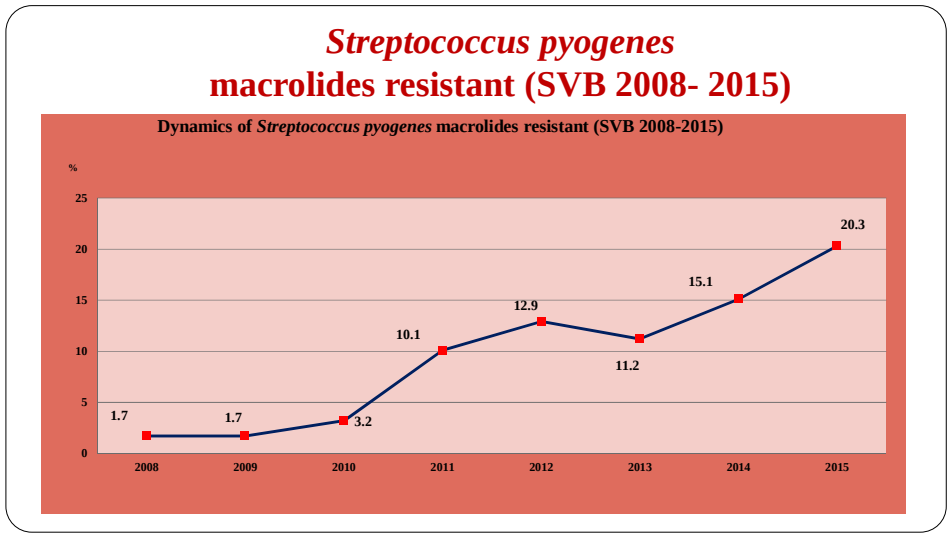
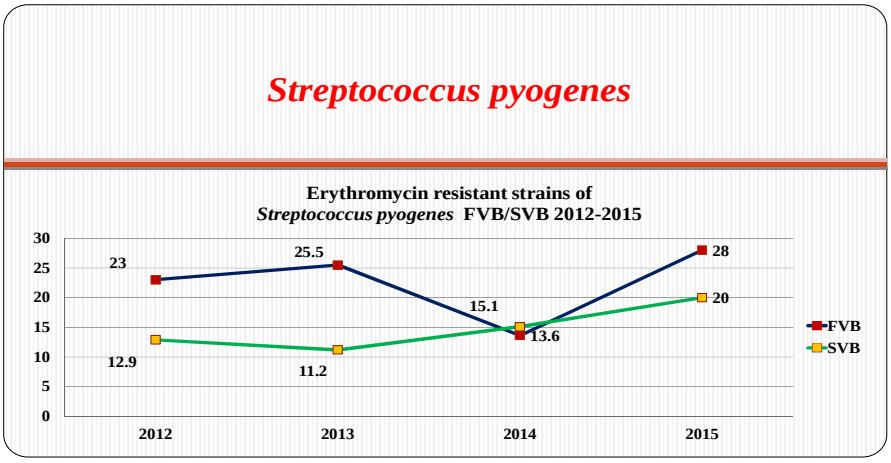
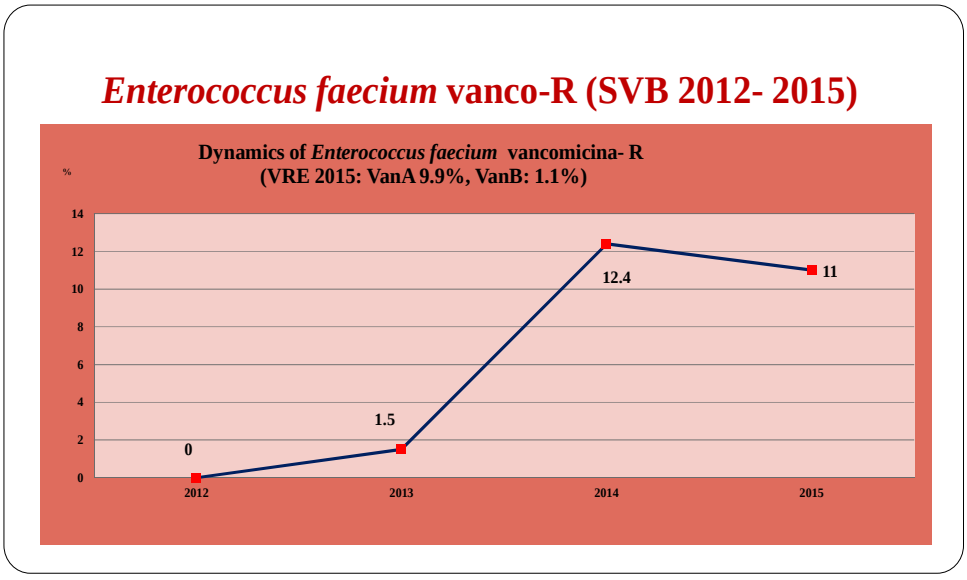


Fig. 4:



3. *Enterococcus faecium* strains isolated from urinary tract infections, showed 0% resistance to vancomycin in 2012 and 1.5% between 1999 and 2013. Patients had a history of complex urinary pathologies, with repeated hospitalizations in urology wards, other surgical specialties, ICU, etc. A significant growth was recorded in 2014 of 12%, and in 2015 of 11%.

Fig. 5:



4. The incidence of E. coli strains and beta-lactamase-producing *Klebsiella pneumoniae* with extended spectrum / ESBL: Between the years 2000 to 2015 a progressive increase in the incidence of *E. coli* strains producing ESBL was recorded at "Dr. V. Babes" Hospital for Infectious and Tropical Diseases, from 3.2% in 2000 to 27% in 2013, and 21% in 2015. This situation is explained by the increase in number of patients with multiple MDR bacterial infections transferred from ICU and surgical services from other hospitals to “Dr. V. Babes”, for treatment with cephalosporin class III and the established ICU department in our hospital. *E. coli* ESBL(+) from ambulatory patients experienced a 7.3% isolation rate in 2014 and 6.3% in 2015.

Fig. 6:

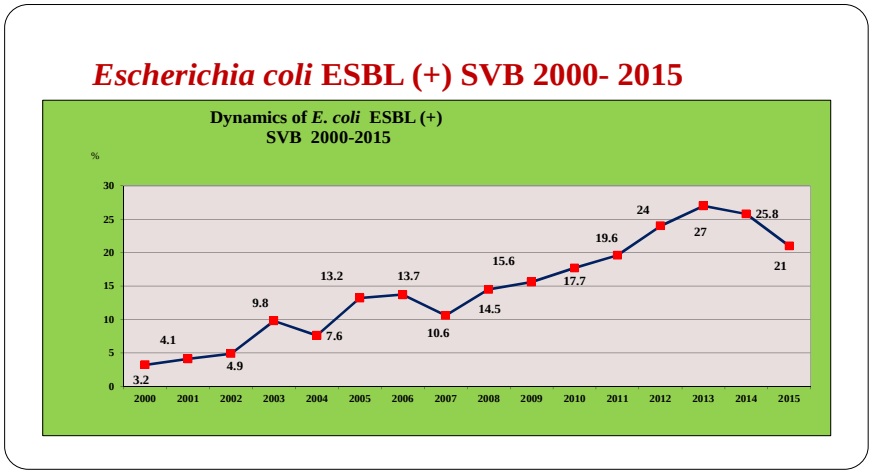
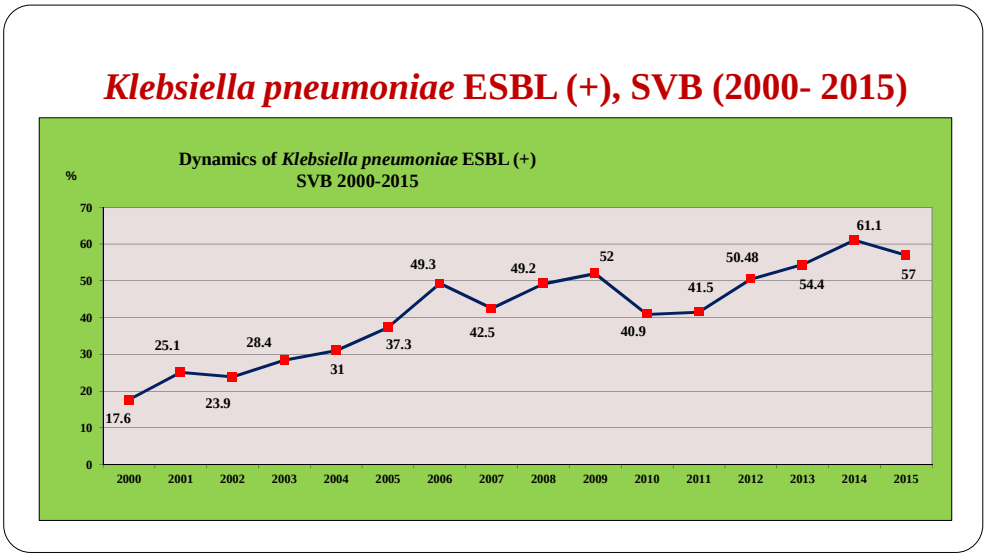
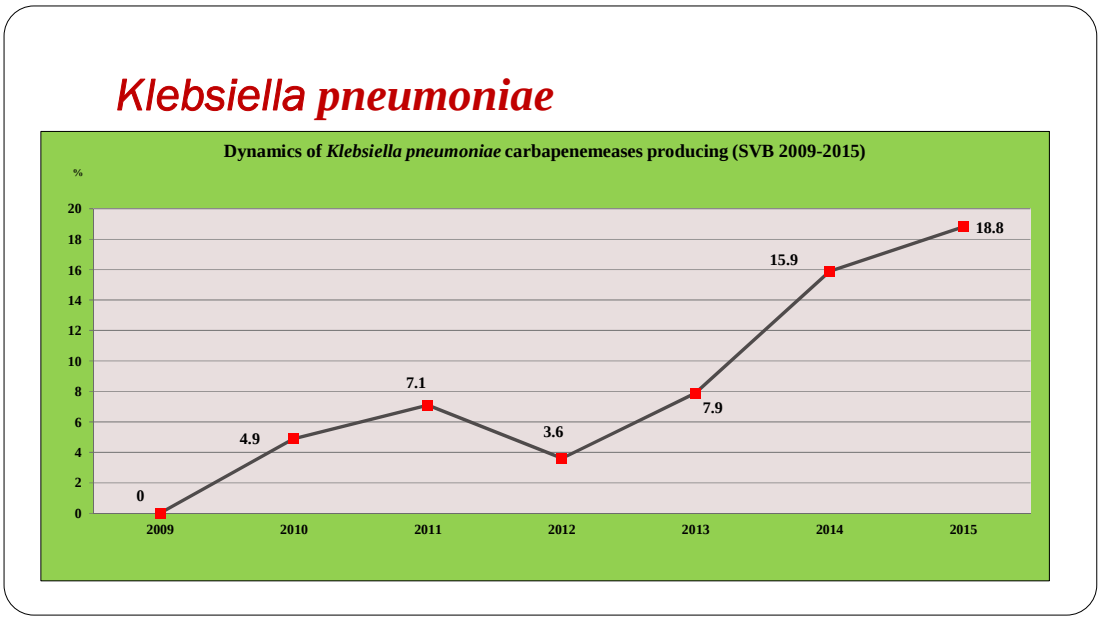


Fig. 7:



Looking at *Klebsiella pneumoniae* extended spectrum beta lactamases / ESBL producing strains isolation rate, it has increased progressively from 17.6% in 2000 to 61.1% in 2014 and in 2015 was 57%. Isolates originated mainly from urine from patients with multi-admissions in urologic surgery wards, transferred to SVB for strengthening antibiotic treatment of complicated urinary tract infections. *Klebsiella pneumoniae* ESBL (+) recorded a rate of isolation in ambulatory patients of 15.7% in 2014 and 12.5% in 2015, significantly lower than in St. Harbath (Geneva): isolates of *E. coli* ESBL (+) come predominantly from food source, while strains of ESBL-producing *Klebsiella pneumoniae* were mainly nosocomial (poor hygiene of hands, surgical wards).

Fig. 8:



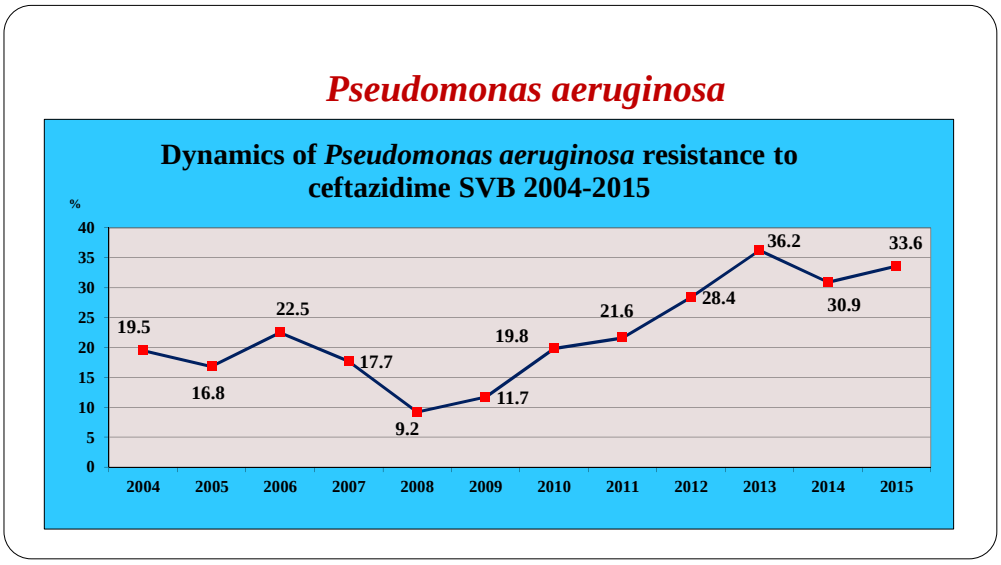
Carbapenems-resistant *K. pneumoniae* isolated strains were 0% in 2009, and increased to15.9% in 2014 and 18.8% in 2015. In 2015, carbapenemases types identified by phenotypic and genetic methods were 35 / OXA-48, 8 / KPC and 21 / MBL (NDM-1). Large plasmids such as the MCR-1 contain the gene which mediates resistance to colistin, and IncHI associated with *K. pneumoniae* strains NDM-1 encode resistance to phosphomycin.

5. Antibiotic resistance of Gram-negative bacteria- Unfermentative:

A distinctive feature of the species *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (the pathogens particularly responsible for hospital-acquired infections) is their high level of intrinsic resistance to antibiotics, particularly beta-lactams. In addition, of importance is the ability of these species to acquire multiple resistance mechanisms, especially enzymatic mechanism commonly accumulated in the same strain.

In the period 2000- 2015, the resistance of resistant isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* to carbapenems is presented in the tables below:

Fig. 9:



Resistance of *Pseudomonas aeruginosa* strains to III-rd generation cephalosporin's (ceftazidime) was 19.5% in 2004, 36.2% in 2013, and 33.6% in 2015.Resistance to carbapenems (in isolates from patients hospitalized in SVB) recorded an upward trend: 23.3% in 2004, 37,9% in 2015. *Pseudomonas aeruginosa* strains isolated from patients in ambulatory showed a resistance rate to carbapenems significantly lower: 14% in 2014 and 8.4% in 2015.

Fig.10:

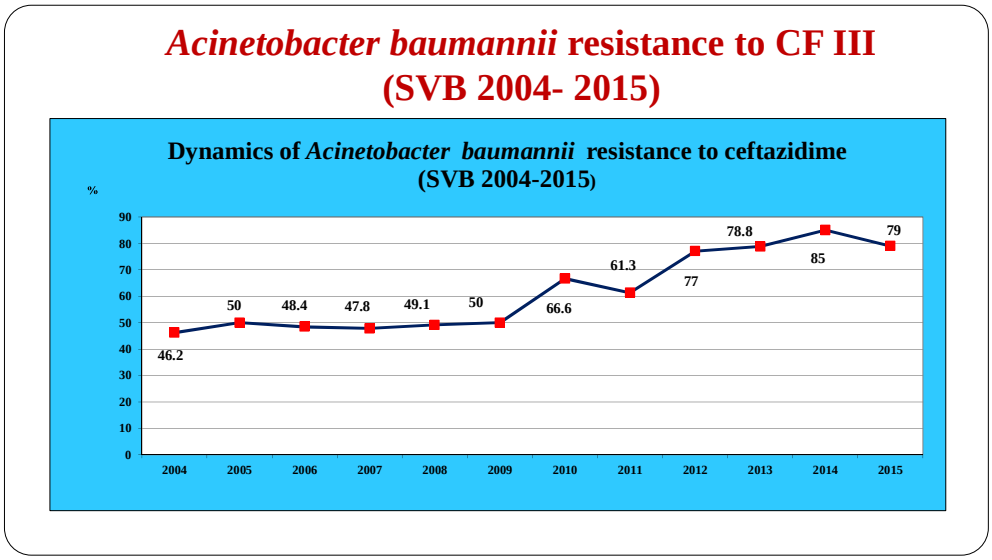
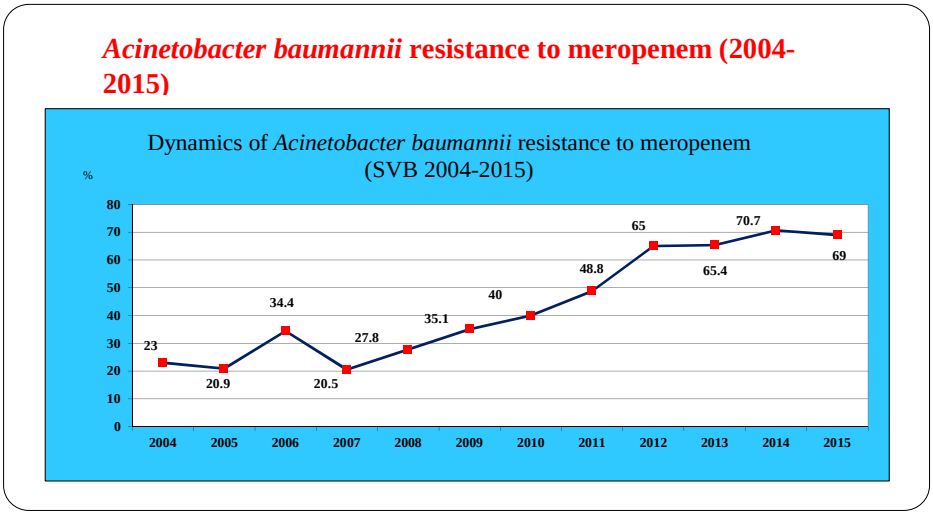


Fig.11:



Isolates of *Acinetobacter baumannii*, from respiratory infections, skin, urine, or from patients transferred from other hospitals to strengthen antibiotic treatment, showed significant resistance to III-rd generation cephalosporin and carbapenems (colonized patients, nosocomial infection).

Conclusions, comments:

Antibiotic resistance of bacteria is a major challenge for public health. Therapeutic solutions are extremely limited: polymyxins class of antibiotics is most commonly used in treatment regimens, with carbapenems, tigecycline, phosphomycin, aminoglycosides etc., depending on resistance mechanisms identified in vitro.

Proposed measures to prevent the occurrence and spread of antibiotic resistance in hospitals and communities are:

- Infection Prevention: Basic personal hygiene measures and collective control of nosocomial infections, vaccination (reduces the need for antibiotics);
- Reducing the use of antibiotics (in human and veterinary medicine, animal breeding and farming, environment);
- The introduction of new antibiotics to treat MDR bacteria;
- Proper use of antibiotics;
- Complementary pathways or alternative therapy;
- Implementation of national and international structures of antibiotic resistance surveillance and decision on urgent measures for lowering it;
- Application of new technologies and advanced research methods to study the molecular and genetic mechanisms of the acquiring of bacterial resistance to antibiotics, providing a huge potential for the discovery of new molecules or the modification existing ones. Many of the old natural products with antibiotic activity may be reconsidered in terms of chemical and microbiological treatment;
- The pairingand combination of antibiotics represents a promising idea in achieving success in treatment of emerging and re-emerging diseases;
- The solution for reducing the incidence of MDR bacterial infections would be to preserve precious resources represented by the "reserve antibiotics", which should be used only in extreme life saving situations;
- The necessary responsible involvement of all stakeholders: patients (through medical education, proper self-medication), doctors (minimizing abuse and under-dosing, minimizing antibiotic use as anti-pyretic medications in viral infections) as well as stakeholders in agriculture and food and animal industries, in order to preserve effective antibiotics for the generations to come;

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VISCERAL LEISHMANIASIS IN A ROMANIAN IMMIGRANT IN SPAIN

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Key words: visceral leishmaniasis, mediterranean, immigrant

Background: Visceral leishmaniasis is a reticuloendotheliosis parasitic infection caused in humans and animals by the genus *Leishmania* protozoa, transmitted by insects of the genus *Phlebotomus*.

Visceral leishmaniasis is the most serious of the three forms of leishmaniasis that could appear in humans (these are: coetaneous, mucocutaneous and visceral). There are over 20 species of *Leishmania* and over 90 species of Phlebotomus that can transmit the disease. Reported prevalence is around 900,000 to 1.3 million every year, with a declared mortality of 20-30.000 deaths a year.

The geographic endemic area of visceral leishmaniasis is mainly tropical Africa, Asia, and America, but also in the temperate regions of Asia, South America and Europe, where Phlebotomus species employed as vectors live. The endemic area has expanded greatly since 1993, and the number of cases has increased significantly. This growth has been caused by climate change, which allowed the spread of the vector population,but also through population migration from the rural areas (endemic) towards the urban areas, increasing the human container of leishmaniasis (notably by HIV/ AIDS infected population). Also an important man made contribution was the change in the environment through building dams, irrigation systems and wells, and through deforestation. Social conflicts and natural disasters have additionally contributed to the emergence of leishmaniasis in some regions: for example, visceral leishmaniasis in Sudan (100,000 cases in a region with fewer than 1 million inhabitants), skin leishmaniasis in Afghanistan.

The natural reservoir of *Leishmania* is represented by a large number of animals, wild and domestic (mostly dogs), and humans.

For most animals the infection is mild, often asymptomatic, with *Leishmania* persisting in the body for many years.

The exceptionis dogs,who develop a generalized severe and often fatal disease. Depending on the reservoir of parasites there are two types of leishmaniasis: zoonotic, where the reservoir is represented by animals, especially dogs (Mediterranean basin, Latin America), and anthroponotic in which man is the only reservoir and the source of infection (Asia, East Africa).

Visceral leishmaniasis is present over wide territories between 45° north latitude and 32° south latitude.The disease is endemic in 61 countries located in Asia (especially India, Bangladesh, China, Indochinese peninsula), Africa (Maghreb, Central and Eastern Africa), America (mainly Brazil and Central America), but also in South of Europe. 90% of total cases of visceral leishmaniasis were recorded in five countries: India, Bangladesh, Nepal, Sudan and Brazil. In Europe, visceral leishmaniasis is endemic in countries located in southern region of the continent, mostly the Mediterranean basin (Spain, Portugal, France, Italy, Greece, etc.). Sporadic cases of imported cases are reported in other countries (including Romania).

Leishmanias are flagellate protozoan that are part of *Trypanosomatidae* family, genus *Leishmania*. *Leishmania* genus classification is based on phylogenetic characteristics and isoenzyme profile, distinguishing two major sub-genres: sub-genus *Leishmania* (Ross, 1903) and sub-genre *Viannia* (Lainson and Shaw, 1987).

Sub-genus *Leishmania* includes the following species: *L.donovani*, *infantum*, *tropics*, *mexicana*, *killicki*, *aethiopica*, *major,turanica*, *gerbilli*, *arabica*, *amazonensis*, *enrietti*, *hertigi*. Sub-genus*Viannia* includes the species: *L. braziliensis*, *guyanensis*, *naiffi* and *lainsoni*. There are four species of human pathogenic Leishmania (*L. tropica*, *L. donovani*, *L. mexicana* and *L. Viannia*) each with numerous subspecies. All species and subspecies are morphologically identical, and differentiation can only be made in specialized laboratories by identifying specific isoenzymes.

Leishmanias have two evolutionary forms: promastigote shape and amastigote. The promastigote form is present only in the digestive tract of the insect vector. It has an elongated shape (15 - 25μ / 3 - 4μ) and a flagella located at the anterior end.Amastigote form is present in humans and animals. The amastigote has no flagella and is oval or round in shape with a diameter 1 - 6μ.

In most cases, visceral Leishmaniasis is produced by the species *Leishmania donovani*, and *L.infantum*. Rarely, in certain geographical areas, visceral leishmaniasis can be produced by L.tropica (the etiological agent of cutaneous leishmaniasis).

The first cases of visceral leishmaniasis produced by *L.tropica* were described in the 90s in soldiers participating in activities carried out in Saudi Arabia, Kuwait and Iraq, during the operation "Desert Storm". Similar cases produced by *L. tropica* have been highlighted in recent years in India.

All diagnosed cases of visceral leishmaniasis in our country in recent years have been imported cases. The number of cases has increased in recent years due to the large number of Romanian citizens who have move annually to endemic areas located in southern Europe. Many of them live and work in rural areas where the risk of infection is much higher than in urban areas. It is very likely that in future years we will witness an increase in the number of visceral leishmaniasis cases among returning Romanian citizens from these areas.

The reservoir of infection is represented by humans and animals (especially canines). In the Mediterranean region the main natural reservoir is represented by dogs (domestic and wild). It is considered that in this region, 5 million dogs are affected by visceral leishmaniasis. This imposing reservoir continuously sustains the mammal - phlebotomine – man circuit. Parasite transmission from animal to human or from human to human is through phlebotomines. There is the possibility of transmission through infected blood directly (blood transfusions) or indirectly (syringes, medical instruments, etc.). Transplacental transmission is also possible.

Parasites, located in the pharynx of insect vector, reach the skin of the host through the bite of infected phlebotomines. The local macrophages help the conversion into amastigotes, which start growing through scissiparity. Then follows the formation of a small granuloma composed of mononuclear cells, parasitic cells, epithelioid and giant cells (inoculation chancre).Most times, the inoculation chancre has no clinical expression or it manifests as a small papule, and rarely as an extended ulcerative lesion.

From the skin, Leishmania reaches organs rich in reticuloendothelial tissue through blood circulation. Here it continues its intracellular multiplication in the organ’s macrophages. The result is the appearance of lesions in organs, infiltration with mononuclear phagocytes and the organ’s hypertrophy (liver, spleen). In addition to liver and spleen, leishmania parasites also attack the phagocytic mononuclear cells from the bone marrow, lymph nodes, intestine, skin, etc. Sometimes they strike polynuclear cells and even erythrocytes.

Incubation is in average 3 to 6 months, ranging between 10 days to 3 years. Very long incubation periods have also been described, of up to 10 years, but only in people with initial asymptomatic infection which became clinically evident after a while due to immunosuppression.

The site where Phlebotomus stings sometimes develop a rash for a limited period of time (the inoculation chancre).

Disease usually starts insidiously with irregular fever (37.5 to 38.5°C), fatigue, loss of appetite. This state usually last 2 to 3 weeks after infection. Clinical manifestations consist of fever, impaired

general condition, asthenia, moderate hepatomegaly, important splenomegaly, enlarged lymph nodes, mucosal bleeding, extreme pallor, weight loss (often leading to cachexia).

There are also asymptomatic forms of visceral leishmaniasis. Serologic studies conducted in Brazil and Israel in a cohort population have shown a high prevalence of seropositivity for Leishmania, compared with the small number of clinical cases of the disease.

The outbreak of visceral Leishmaniasis in India and in East Africa caused by *L.donovani* is characterized by distinctive changes of the skin. The patients acquire a brown or black coloration of the skin,hence the name Kala-azar (fever-black) for this form of leishmaniasis, and it also involves the appearance nodular formations at face level (Kala-azar post nodules).

If untreated visceral leishmaniasis would progress slowly to death within 6 months to 2 years.

During this period of infection, various complications may occur including secondary bacterial infections, which may lead to the unfavorable evolution of the disease.

Diagnosis is established through the basis of epidemiological data (originally from endemic areas or traveled into these areas, especially in rural areas), clinical (irregular fever, persistent fatigue, hepato-splenomegaly, weight loss) and laboratory investigations.Laboratory investigations reveal pancytopenia (sometimes significant), elevated sedimentation rate (ESR > 100 mm / 1h), hypergammaglobulinemia.

Correct diagnosis is established by evidence and / or isolation of parasites in various pathological products (spleen, liver, lymph nodes, bone marrow) through:

- Direct examination of smears: May - Grunwald – Giemsa stains (cytoplasm of parasites is colored pale-purple and nucleusis red);
- Cultures must be done from bone marrow and blood samples, on specific media N.N.N. (Novy, Mc.Neal, Nicole) or on R.P.M.I. (Roswell Park Memorial Institute);
- Genomic amplification (PCR) with very high sensitivity and specificity.

The differential diagnosis is with malaria, typhoid fever, brucellosis, extra pulmonary tuberculosis and other noninfectious diseases, including hematologic disorders (lymphoma) that are often confused with Leishmaniasis, particularly in non-endemic areas.

Etiological treatment consists of: pentavalent antimony derivatives, liposomal amphotericin, Pentamidine and Miltefosine. There is no vaccine for human use against leishmaniasis.

Methods

We present patient D.M., aged 29, male, who was admitted to our clinic for prolonged fever, chills, impaired general condition, marked fatigue, loss of appetite, weight loss of 7 kg. The symptoms gradually began three months before he addressed our clinic. The patient was admitted to several hospitals, received repeated treatments with broad-spectrum antibiotics and symptomatic medication, without symptomatic relief.

The epidemiological history noted a journey lasting several months to Spain, in the Cordoba region, where he worked at strawberry picking and where he slept outdoors several times.The patient had traveled with his wife, who was asymptomatic.

Clinical examination revealed paleness, marked hepatosplenomegaly and multiple enlarged and painless lymph nodes.

Biological samples showed pancytopenia with Hb values of 7.92 g / dL, WBC number 719 / mmc, 61,900 platelets / mmc, inflammatory syndrome ESR 63mm /1h, fibrinogen 304 mg /dL, ALT 47 IU /L, creatinine 0.8 mg /dL, glucose 82 mg /dL, Na 127mmol /L, K4.3 mmol/L, HBsAg-positive, HIV negative AcAcHCV negative, blood cultures, urine cultures, including *Salmonella typhi* negative, throat swab negative.

Radiological examination showed normal pulmonary imaging. Abdominal ultrasound showed hepatomegaly with LHD of 162 mm diameter, LHS of 87 mm, splenomegaly with length of 265 mm.

Parasitological examination of bone marrow biopsy revealed the presence of intra and extracellular common forms of Leishmania amastigote.(fi. 1, 2)

The patient received amphotericin B deoxycholate in treatment dose of: 1mg (test) 20mg-30mg - 10mg-40mg-50mg, followed by 50mg i.v / day, 15 days.Evolution under treatment was slowly favorable, without fever, hematological improvement and regression of inflammation.

He was discharged cured. He never returned for a check-up and was later lost in surveillance.

Fig. 1

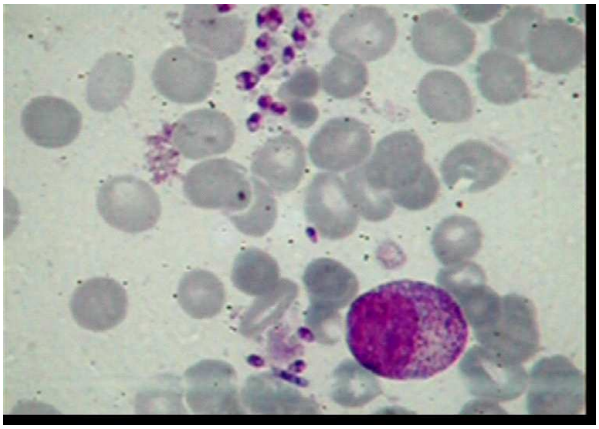
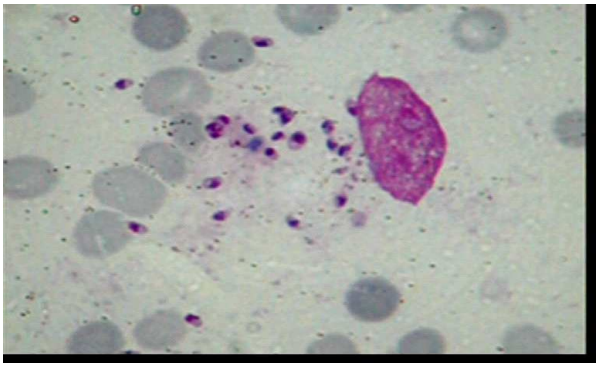


Fig.2



Discussions

Although the Mediterranean is known as endemic of leishmaniasis with high risk of transmission, the certain diagnosis of this patient was delayed for several months, even in an endemic area. Besides the fact that he had lived in an endemic area for an extended period of time, the patient had additional risk factors represented by epidemiological work outdoors, where he was probably exposed to the action of the insect vector. In these circumstances, we believe that a correct history with an assessment of the degree of risk represented by the above mentioned factors would have lead to an early diagnosis and a correct treatment, as well as avoiding a severe form of the disease and prolonged patient suffering.

Therefore we believe that addressing such cases must be done by obtaining all epidemiological details, especially in the context of current movement of immigrant populations and the increase in extended travels across Europe and beyond.

Etiologic diagnosis of these cases should be performed in clinics with experience where patient should be quickly referred, given that the disease can potentially evolve to severe if there is an incorrect and ineffective diagnosis and treatment method.

Alternative etiological treatment was used, and the first choice was not antimony salts because they are not available in Romania. After this particular case, efforts were made to establish a medication fund reserve for these cases at "Dr. Victor Babes"Hospital for Infectious and Tropical Diseases.

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HEPATITIS E: A ONE HEALTH APPROACH IN NORTH-EASTERN ROMANIA

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Key words: hepatitis E virus, zoonotic, spreading

Hepatitis E virus (HEV) is responsible for epidemics and endemics of acute hepatitis in humans, mainly through waterborne, foodborne and zoonotic transmission routes. HEV is an important enterically transmitted human pathogen with a worldwide distribution. A number of animals are known to serve as the natural hosts and reservoirs for HEV. The virus has been genetically identified from rat, wild boar, domestic swine, mongoose, rabbits, chickens, ferrets, cutthroat trout, bats, and deer [1]. Anti-HEV antibodies have been detected in a number of other animal species including cattle, sheep, and goats with the potential to carry novel strains of HEV [2]. With the advance of modern molecular biology techniques such as metagenomics and pyrosequencing, it is expected that the host range of HEV will expand and novel strains of HEV will be identified from other animal species in the near future.

Human and swine hepatitis E virus are classified in the *Hepeviridae* family as a separate *Orthohepevirus* genus - *Orthohepevirus A* species [3]. Like most enteric viruses, HEV is a relatively stable particle, resistant to gastric juice and bile salt, which explains its survival in the intestinal environment. The genome is represented by a single-stranded positive sense RNA molecule of 7.2 kb length which contains three open reading frames (ORF). Based on the ORF2 nucleotide sequences analysis in mammals, four major genotypes were defined. Genotypes 1 and 2 do not infect swine, while genotypes 3 and 4 are zoonotic [4]. Hepatitis E virus replicates in the cytoplasm of hepatocytes and is excreted in faeces [5]. HEV is enterically transmitted and causes an acute and generally self-limiting infection of the liver but with a higher mortality in general than for infections with hepatitis A virus. HEV is unique among the hepatitis viruses because of a high mortality during pregnancy where the rate is up to 30% [6].Isolates from chicken are included in *Orthohepevirus* B species [3]. Avian HEV was associated with big liver and spleen disease inAustralia [7] and hepatitis-splenomegalysyndrome (HSS) in North America [8]. Subsequently avian HEV infection has been associated with disease outbreaks in chicken flocks worldwide.

Hepatitis E virus (HEV) is endemic in developing countries, but reports of infection with this virus in industrialized countries are becoming more frequent [9]. Most of these are due to HEV genotype 3, and have been related to a high mortality rate, mainly in those patients developing acute-on-chronic liver failure[10,11]. In many countries, including Romania, the incidence of HEV infection has not been examined and zones with poor sanitation should be assumed to have a high risk for endemic HEV infection and disease. In developed countries, sporadic clinical HEV is rare, and epidemics have not been reported. Industrialized countries, such as Canada, Europe, Japan and the USA were previously thought to be exempt from HEV, with a limited number of cases reported only in people who had travelled to endemic areas of the world. However, more recent studies have documented a number of sporadic cases in developed areas, including the USA and Europe, among patients who had no history of travelling to hepatitis E endemic countries [12]. Furthermore, a high anti-HEV seroprevalence (in some cases reaching 20%) has been detected in a significant proportion of healthy individuals of non-endemic countries [12]. Although acquisition of infection in industrialized countries was typically believed to occur only among travelers to endemic regions, growing evidence indicates that cases in industrialized countries may be autochthonous (locally acquired) and may represent porcine zoonosis [9]. Hepatitis E was diagnosed in industrialized countries, where it was demonstrated that the genomic sequence of

isolated HEV strains was more related to swine HEV prevalent in swine population from the same region [13]. The existence of a zoonotic reservoir was demonstrated by virus isolation from wild and domestic animals: pigs, cattle, chickens, sheep, goats and rodents [14,1]. The first HEV animal strain was isolated from pigs in the United States [15].

The epidemiology of HEV is more complex than was initially appreciated, and many features remain unexplained, though zoonosis seems to be the main way of transmission. Some cases of acute transfusion-transmitted hepatitis E infections, have led to an increasing number of publications reporting the prevalence of serum Ig G antibodies to HEV (anti-HEV) in blood donors in western countries. These rates oscillate between 4.7% in Scotland [16] to up to 52% in adults from Midi-Pyrénées [17]. However, regarding industrialized countries, it should be stressed that important differences in the prevalence rates have been described and related to age, geographic region and even the anti-HEV assay used. Autochthonous hepatitis E infection, caused by genotype 3 is recognised as an emerging infectious disease in developed countries.

Previous investigation on HEV infection in Eastern Romania revealed 12% anti-HEV IgG prevalence (3 out of 25) in patients diagnosed with hepatitis B or C [18] and 5,9% anti-HEV IgG seroprevalence (4 out of 67) in general population [19]. Also, Voiculescu et al reported in 2010 the HEV seroprevalence of 12.5% in very low risk population and 13,98% in low risk population [20].Studies made on human hepatitis E infection in North-Eastern Romania highlighted that in 2011 a 17,14% seroprevalence (12/70) was estimated, whereas in 2012 a 12,82% seroprevalence (10/78) was recorded. Anti-HEV IgG antibody in subjects without clinical signs of hepatitis was used as an epidemiological tool to measure exposure to this virus. These results revealed that HEV infections in the Eastern area of Romania affect middle-aged adults [21]. A recent study on a group of 51 subjects (with or without signs of clinical disease) from the Infectious Diseases Hospital “Sfanta Parascheva” Iași was undertaken between February and June 2015. The results of the investigation showed that 15.7% of the pacients were identified as positives for IgG anti-HEV antibodies. The seropositive patients were on average older than the seronegatives (43 vs 33 years, p=0.08). Five of them were males and the sex ratio (M/F) was higher among the seropositives (1.7 vs 0.3, p=0.07). There was no significant difference between the two lots regarding their living conditions (urban or rural), but a more frequent contact to farm animals (pigs) was noted in the seropositives. Two of the seropositives had symptoms and liver enzymes elevations typical of acute hepatitis (without jaundice or other viral cause identified), one of them being a young pregnant woman. No difference in the average ALT and serum bilirubin levels were noted in the asymptomatic patients, according to their IgG anti HEV status.Two out of eight seropositives vs 6/43 seronegatives had prior unexplained self-limited hepatocytolitic syndromes [22].

The disease is currently considered an emerging zoonosis. The first direct evidence of a possible zoonotic transmission of HEV was provided in Japan in 2003, when cases of hepatitis E were caused by the ingestion of uncooked meat or organs from pigs, wild boar or deer. Studies on anti-HEV antibodies detection have demonstrated that people working in contact with swine or wild boar have a higher risk of infection than normal blood donors [23]. Pig handlers such as veterinarians, breeders, and farmers in China, Thailand, The Netherlands, Sweden, Moldova, and the United States were more likely seropositive to swine HEV [24]. In Sweden, 13% of pig breeders were positive for HEV antibodies. In The Netherlands, 11% of swine veterinarians were positive in comparison to 6% of non-swine veterinarians and 2% of the general population. In Moldova, 51% of swine farmers were positive in comparison to 25% of non-swine occupations [25].

Accumulating data indicates that human HEV infections are mediated by the consumption of uncooked or undercooked animal meat or foods made with swine organs such as liver. A study reported that the full genome of HEV isolated from wild boars had a nearly identical sequence (99.7% identity) to that of HEV isolated from wild deer and patients who contracted HEV after eating raw deer meat. These data imply that pigs, wild boar and wild deer are an important source of HEV infection in humans [26].

Recently, European countries have also reported sporadic cases of HEV infection. Consumption of raw seafood, pork liver sausage, and exposure to wild boars are proposed as major risk factors associated with HEV infection in Italy [27]. One of the risk factors for HEV infection in France is the ingestion of raw pork liver sausage, which has been reported to contain infectious HEV particles [28]. Wild boar meat consumption was positively associated with HEV infection in a case-control study in Germany. Similar investigations of pork production chains in the Czech Republic, Spain, and the United Kingdom revealed detectable, infectious HEV at both processing locations and point of sale [29]. Deer have been implicated both acting as animal reservoirs for HEV and acting as vehicles for human infection. In Hungary, the European roe (*Capreoluscapreolus*) deer was implicated as a reservoir species for HEV, and in The Netherlands 5% of red deer (*Cervuselaphus*) were also found positive for antibodies to HEV [23]. Rabbits may serve as reservoir hosts for HEV transmission to humans given the genetic identification of zoonotic genotype 3 strains of HEV from rabbits in China, the United States and France [30]. Rabbits are susceptible to experimental infection by genotype 4 human HEV, and the infected rabbits developed viremia, seroconversion to anti-HEV, and faecal virus shedding. The rabbit HEV is genetically and antigenically closely related to other mammalian HEV [31]. The capsid protein of the Genotype 3 rabbit strain of HEV was capable of cross-reacting with antibodies from other strains of HEV including rat, swine, human, and chicken.

Rats can be infected with seemingly rat specific HEV or with genotype 3. Serological investigations have suggested that humans may be infected by rat specific HEV. It is likely that genotype 1-4 infecting rats can also infect humans. Because *Rattusnorvegicus* is a synanthropic species, humans and rats live in close proximity. Rats are well known to transmit pathogens to humans and other animals. Their high prevalence and propensity to carry pathogens make them a potential reservoir for human pathogens including HEV [32]. Indeed, according to previous surveys, antibodies against HEV are highly prevalent in rats and HEV RNA has been detected in rats (*R. norvegicus* and other rat species) in Germany[33], USA, Vietnam, Denmark, China and Indonesia. Most HEV strains found in rats were of the rat specific type (species *Ortohepevirus C*), but genotype 3 RNA (species *Ortohepevirus A*) has also been detected. The rat strain of HEV was identified in wild Norway rats from Hamburg, Germany with 59.9% and 49.9% nucleotide sequence identity with known human and avian HEV strains, respectively [33]. Therefore, it is important to further clarify the role rats may have as a reservoir for human HEV infections.

Swine HEV strains have a worldwide distribution, and infection in pigs from both commercial farms and wild populations is almost ubiquitous. Swine HEV has been shown to cross species, and the close genetic relationship between swine HEV and strains isolated from some patients in the United States, Taiwan, Korea, and Japan is consistent with a zoonotic source of these indigenous infections, although swine may not be the only reservoir. As such, HEV infection should be considered a possibility in patients with acute hepatitis who do not have a relevant travel history or markers of other hepatitis viruses.

In Romania the data available regarding hepatitis E virus infection in animals highlighted that HEV is endemic in swine population.In 2010, Aniță A. et al, in a seroepidemiological study on hepatitis E virus infection in swine in Botoșani County [18] revealed the presence of anti-HEV antibodies (IgG) in 22,66% household pigs (17 out of 75). In 2014, Aniță A. at al, revealed HEV circulation in pig farms. Based on phylogenetic analysis of the ORF2 sequence the Romanian swine HEV isolates were included into genotype 3. This was the first study showing HEV to be present in Romanian pig herds and that the human population is exposed [21]. The HEV infection in wild boar population was detected for the first time in 2009 when a seroprevalence of 4,44% was detected [34]. Recently two studies highlighted the possible risk of wild boar as reservoir of HEV [35, 36] in four Counties from Eastern Romania (Buzău, Vrancea, Bacău, Iași), where the seroprevalnce of IgG anti-HEV antibodies varied from 9,61% to 11,39%.

In the last decade, HEV infections took on greater significance for public health. Considering the current statistic published by the Robert-Koch Institute on notifiable infectious diseases, hepatitis E cases have been increasing continuously during the last decade. In Romania, compared with other European Union countries such as France or Germany, hepatitis E virus infection in animals and humans is not

subject of current surveillance and diagnosis. The relevance of each source for public health risk in Romania is currently unknown.

Prioritizing these sources by use of risk assessment can guide further research and aid the assessment of the animal importance in HEV exposure for humans in Romania. Considering the zoonotic risk arising from animals, the importance of further investigations and intensive education of the population become evident. It is especially crucial to raise the awareness of HEV in all individuals in daily contact with susceptible animals, such as veterinarians, farmers, hunters or slaughter house workers.

Zoonotic diseases are difficult to control, particularly because of their animal reservoirs, but the risk reduction requires multidisciplinary teams and a unified concept of medicine in humans and animal species.

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RARE CASE OF DIROFILARIASIS IN A PATIENT OUTSIDE THE ENDEMIC ZONE

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Key words: Dirofilaria, nonendemic, human

Background

Dirofilariasis is a vectorial transmitted disease, very rarely found in humans. The first case of dirofilariasis in humans was recorded by Victor Babes in 1880. The past years have seen a rise in the number of cases of dirofilariasis in different countries of the world. In Romania, there have been rare cases of conjunctival and subcutaneous types.

The etiological agent is represented by a nematode from the family Onchocercidae, genus Dirofilaria. The most frequently involved species of Dirofilaria involved in human pathologies are the D. Immitis associated with pulmonary involvement and D. repens characterized by subcutaneous and conjunctival involvement.

The vectors of transmission are represented by mosquitoes which also populate our country, like the species Anopheles, Aedes and Culex. Humans are an accidental host, and in the human body the inoculated larvae don't reach the necessary level of maturity for the appearance of microfilaria, which raises the difficulty when it comes to a correct diagnosis.

Methods

We present a 46 years old patient from Bucharest, admitted in our hospital in September 2011; prior, he was examined at the Emergency Ophthalmology Hospital complaining of a sensation of a mobile foreign object at periocular level, with transient presence at palpebral and conjunctival level, as well as local erythema and discomfort. The described simptomatology had started 2 months prior. The ophthalmological consult finds no palpebral tumor, and a normal retinal exam, and the patient is sent to Victor Babes Infectious and Tropical Diseases Clinical Hospital for additional investigations.

The patient had not traveled recently, and did not own pets.

Clinical exam

Good general state, no fever, discreet left palpebral edema, conjunctival hyperemia, normal cardiac and respiratory values, soft abdomen, liver and spleen in normal limits, alert and oriented, no neurological focal signs. Pulmonary X-ray: No images of acute or evolving pleuropulmonary lesions. Heart, aorta in normal limits.

Routine hematological and biochemical values were in normal ranges. Serological testing for Toxocarosis, both IgM and IgG, were negative. Parasitological blood tests (blood smear, Giemsa coloring) did not confirm microfilaria.

Taking into account the fact that during hospitalization the patient did not feel the mobile sensation previously mentioned, the patient was discharged with the recommendation to closely observe

the migrating formation and to present in the emergency ophthalmology services in case of reappearance of the ocular formation for surgical extraction of the parasite.

Soon after discharge, the patient accuses the onset of the anterior symptoms and present in the ER of the Emergency Ophthalmology Hospital where a live roundworm was extracted from her left subconjunctival space.

Parasitological exam

Macroscopic examination: white, thin, cylindrical roundworm with a length of aproximately 13-14cm and a thickness of 0.2cm

Microscopic examination identified a thick cuticle with transversal striations, and rounded anterior and posterior ends.

On the basis of its length and morphology, it was identified as Dirofilaria sp.

Discussion

The presented case was diagnosed as a case of subconjunctival dirofilariasis on the basis of the morphological parasitological examination. The definitive diagnosis, with detection of the specific species is harder to achieve through morphological methods and is achieved through molecular techniques, like PCR, which are not routinely available.

The rarity of dirofilariasis in Romania as well as the doctors' lack of experience when it comes to this disease lead to a delay in diagnosis in this case. The simple and quick alleviation of the symptoms, even if repetitive, equally determined a setback in the diagnosis. Both the diagnosis and treatment of subcutaneous or subconjunctival dirofilariasis involve surgical extraction of the parasite. The association of antiparasitic medication is not necessary.

The source of infection is represented by local mosquitoes, and the natural host is most probably canine, considering that the patient lives in Bucharest near a lake, in a neighborhood with an important population of stray dogs.

Conclusions

For an accurate dirofilariasis diagnosis in humans, it is firstly necessary for medical staff to be aware of this disease. Subcutaneous/subconjunctival dirofilariasis should be considered a differential diagnosis for any mobile or fixed subcutaneous nodule or for any simptomatology suggestive for cutaneous migrating larvae.

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