ANALYSIS OF CIRCULATING STRAINS, CAUSING INVASIVE LISTERIOSIS IN BULGARIA FOR TEN YEARS, 2010-2019

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ABSTRACT

Listeriosis is a zoonosis with multiple mechanisms of infection and multiple organ symptoms, severe course and high lethality. An increasing incidence of listeriosis has been reported in several European countries in recent years. A limited range of Listeria strains is responsible for most outbreaks occurring in different countries. The aim of the study is to monitor the spread of the Listeria strains causing invasive listeriosis in Bulgaria for the period 2010-2019 and to analyze the etiological structure of the infection in different hospitals in the country. A total of 56 Listeria strains from 17 hospitals were investigated for confirmation of species and serogroup. The materials were isolated from haemocultures, amniotic fluids, cerebrospinal fluids, anal and throat secretions and two tests were used. Confirmed strains isolated from the clinical samples for the study period belonged to 4 serogroups of Listeria. *Listeria monocytogenes serogroup I* was detected in 28 (50%) of the samples, *Listeria monocytogenes serogroup II* – in 43%, *Listeria innocua* –in 5% and *Listeria welshimeri* – in 2% of the samples. The strains isolated from cerebrospinal fluid predominated (52%) and those isolated from haemocultures were 36%. Nineteen of the 29 isolated strains from cerebrospinal fluid belonged to serotype *4b*. *Listeria monocytogenes serogroup I* was detected in 70% of haemocultures. *Listeria innocua* and *Listeria welshimeri* were detected in cerebrospinal fluid. Serotype *1/2a* was found in six hospitals and serotype *4b* - in five hospitals. The majority of isolated strains were from newborns:12/56 (21.43%). Serotype *1/2a* was detected in 12 hospitals and serotype *4b* in 11 hospitals. The largest variety of strains was found in Plovdiv, UMBAL "Sv. Georgi". The data confirmed a steady trend in the spread of certain listeria serotypes in each hospital over the years. Screening at-risk groups, mainly women of childbearing age, is recommended in order to limit the risk of listeriosis in the future.

Keywords: *Listeria, serogroupes, hospital strains*

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INTRODUCTION

Listeriosis is a zoonosis with multiple mechanisms of infection and multiple organ symptoms, severe course and high lethality (17). The infection is defined as zoonosis of increasing medical, social and economic importance due to its severe course, high mortality and specific diagnosis. *Listeria monocytogenes* is the causative agent of human listeriosis, a potentially fatal food-borne infection. Clinical manifestations range from febrile gastroenteritis to more severe invasive forms, including sepsis, meningitis, thrombencephalitis, perinatal infections and abortions (21). An increasing incidence of listeriosis has been reported in several European countries in recent years. These increases reflect a predominantly higher rate of bactereamic listeriosis in patients ≥65 years of age and are unrelated to geographical location, gender, ethnicity, socioeconomic factors, or infectious serotypes (1).

In Europe, invasive listeriosis is an infection of great concern to public health due to its clinical severity (hospitalization rate > 90%) and high fatality rate (20% to 30%). The infection is characterized with low incidence (0,4 $\%_{ooo}$) as compared to salmonellosis and campylobacterios, food-borne infections with the highest incidence in all European countries (23.7$\%_{ooo}$ and 45.6$\%_{ooo}$ respectively) (6). Statistically significant increasing trends in listeriosis notification rates were noted in Austria, Denmark, Hungary, Italy, Spain and Sweden from 2005 to 2009 (6). One of the deadliest outbreaks from food contaminated with *L. monocytogenes* (melons) was reported in

the United States in 2011. Using *in vitro* and *in vivo* assays, the two isolated strains LS741 and LS743 were shown to differ significantly from the ordinary laboratory strain 10403S. These strains exhibited increased virulence characterized by higher brain colonization (12).

Over the last years, many reports from European countries have shown an increasing rate of listeriosis in older age while the pregnancy-related cases remained stable (13,14). *L. monocytogenes* is a genetically heterogeneous species, with a small number of strains (serotypes 1/2a, 1/2b, 4b) implicated in human listeriosis. However, a limited range of strains is responsible for most blasts occurring in different countries. For example, from May 2015 to March 2016, an outbreak due to *Listeria monocytogenes* serotype 1/2a and clinical pulsotype never previously isolated in Europe occurred in central Italy (4). In the Czech Republic and Poland, an elevated number of listeriosis patients after salmon consumption were recorded, with prevalence of serotype 1/2a (11). Multinational blast from *Listeria monocytogenes* sequence type 6 infections related to ready-to-eat meat products was announced in Italy in 2019 (5).

According to the latest annual ECDC epidemiological report, five individual member States of the European Union reported markedly increasing trends of listeriosis over the period 2013-2017 (Germany, Italy, Netherlands, Poland and Spain) (7). In Bulgaria, confirmed cases of listeriosis for this period range from 0.10 to 0.49 per 100 000.

The aim of the study was to monitor the spread of Listeria strains causing invasive listeriosis in Bulgaria for the period 2010-2019 and to analyze the etiological structure of the infection.

MATERIAL AND METHODS

A total of 56 *Listeria* strains were investigated in the NRL "Vector-borne infections, listeria and leptospires" for confirmation of the species and the serotyping. They were sent from 9 hospitals in the country and 8 hospitals from Sofia town. The resulting strains were isolated from haemocultures, amniotic fluid, cerebrospinal fluid , and anal and throat secretions. Two tests were used for strain confirmation: 1) Himedia Latex test kit (Hilisteria), Germany 2) Api Listeria (Biomerieux), Australia.

RESULTS

Listeriosis is defined as a zoonosis with increasing medical, social and economic importance, due to its severe clinical course, high mortality and specific diagnostics - serological and cultural. The incidence of listeria infection in our country is very low as compared to other infectious diseases. In recent years, it is approaching the average values for Europe(0.47 per 100 000). In Canada approximately 0,4 listeriosis cases were reported per 100 000 , in Greece - 0.3 and in Sweden - 7.5 per 100 000 (8,15). In Bulgaria, the incidence of listeriosis for the period 2010-2019 was wavy (Fig. 1). Four morbidity peaks were observed: in 2012, 2014, 2017 and 2019. The highest incidence was registered in 2019. This was the highest incidence in the country for a period of 20 years (for example, the incidence in 2001 was 0) (25).

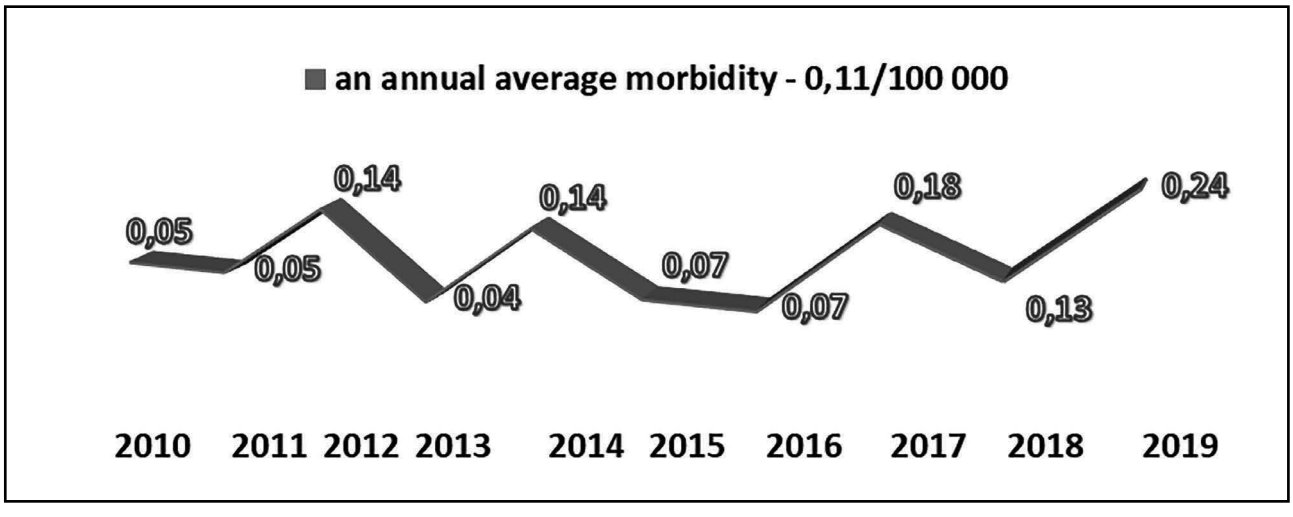

Figure 1. Average morbidity per 100 000 for the period 2010-2019.

Morbidity shows a steady trend towards an increase in the number of patients with listeriosis in Bulgaria and correlates with the trends of the epidemic process in the world and Europe in particular.

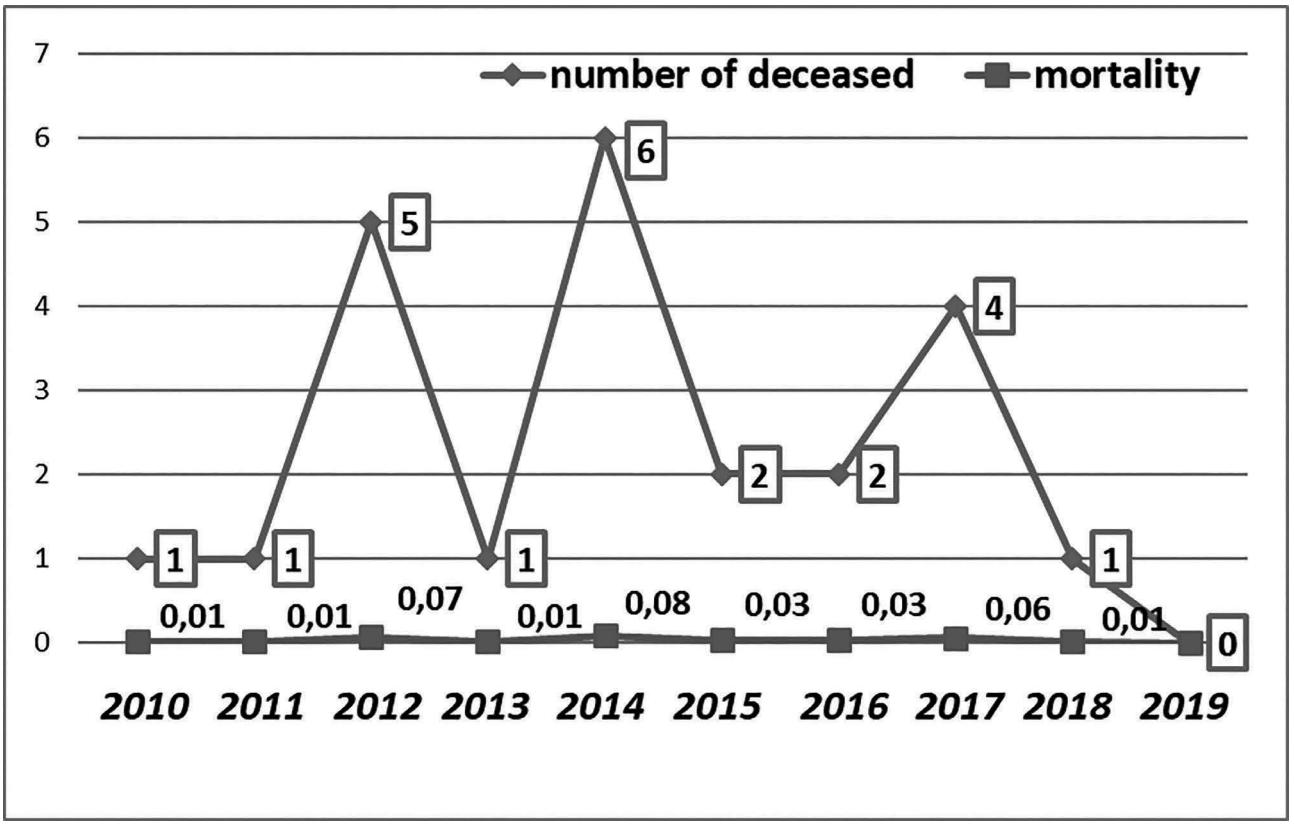

Figure 2. Average mortality per 100 000 for the period 2010-2019.

Fig. 2 presents data of mortality and number of deaths from listeriosis in the study period. Despite of the better detection of listeriosis, the issue of persistently high mortality and letality remains in recent years. The highest mortality rate was registered in 2014, when 6 patients with listeriosis died (0.08 % 000).

Serotyping of the isolated Listeria strains from clinical samples for the study period revealed 4 serotypes (Fig. 3). (28/56) *Listeria monocytogenes I serogroup 1/2a serotype was detected* in the highest percentage of cases, 50%, and - *Listeria monocytogenes II serogroup 4b serotype* - in 43%. *Listeria welshimeri* was detected in only one isolate. *Listeria monocytogenes serotype 1/2a* was isolated in almost all years of the study period. The highest number of isolates of this group were confirmed in 2012 and in 2016, 8 and 6 respectively. The largest variety of Listeria strains was found in 2014 (Fig.4). Interestingly, in 2015 and in 2017 only *4b* serotype was found. *Listeria innocua* was detected in 2014 and comprised 5% of the isolated strains.

The number of isolated Listeria strains is presented in Table 1. Those isolated from cerebrospinal fluid predominated (52%), followed by those isolated from haemoculture (36%) (Fig.5). Only one strain was isolated from anal secretion and one - from abdominal punctat (2%). 19 of the isolated strains from cerebrospinal fluid belonged to *Listeria monocytogenes II serogroup 4b serotype* (19/29) and 7 - to *Listeria monocytogenes I serogroup 1/2a serotype* (Table 1). 70% (14/20) of the isolated strains from haemoculture belonged to *Listeria monocytogenes I serogroup 1/2a serotype*. This serotype was detected from abdominal punctat and anal secretion. Only two strains were isolated from amniotic fluid and they belonged to *Listeria monocytogenes I serogroup 1/2a serotype*.

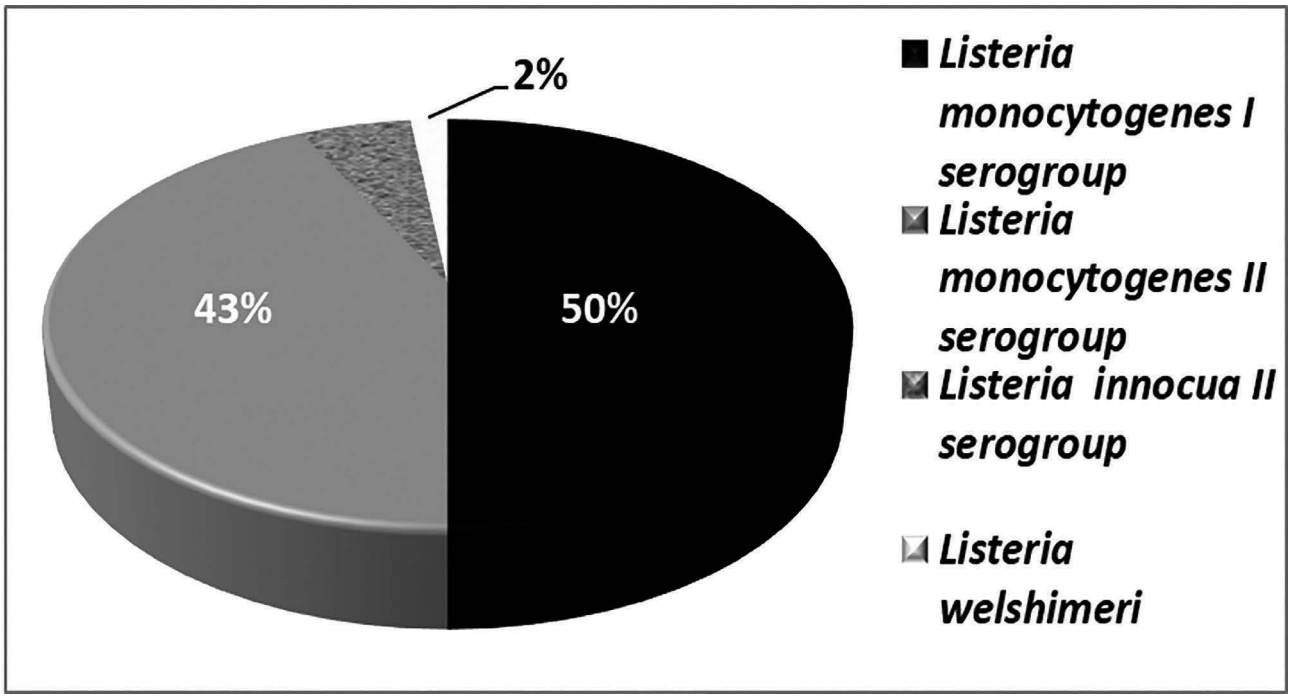

Figure 3. Proportions of Listeria serogroups, 2010-2019.

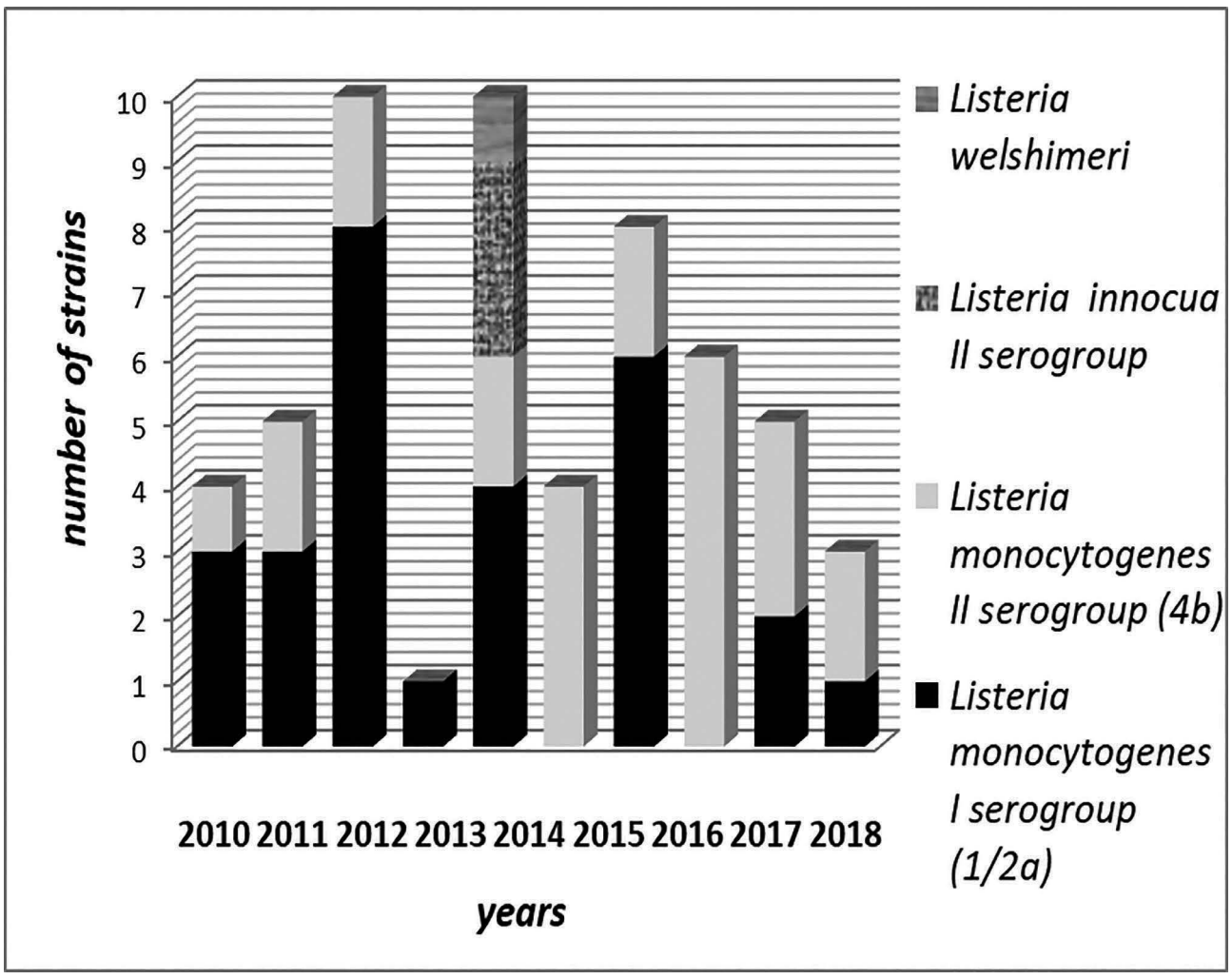

Figure 4. Distribution of isolated strains of Listeria by years.

Table 1. Isolated strains of *Listeria* from the relevant materials for the period 2010-2019

materials / isolated strains	cerebrospinal fluid	haemo culture	amniotic fluid	throat secret	abdominal punctate	anal secretion
Listeria monocytogenes I serogroup	7	14	2	1	1	1
Listeria monocytogenes II serogroup	19	6	1	1	0	0
Listeria innocua II serogroup	2	0	0	0	0	0
Listeria welshimeri	1	0	0	0	0	0
Total	**29**	**20**	**3**	**2**	**1**	**1**

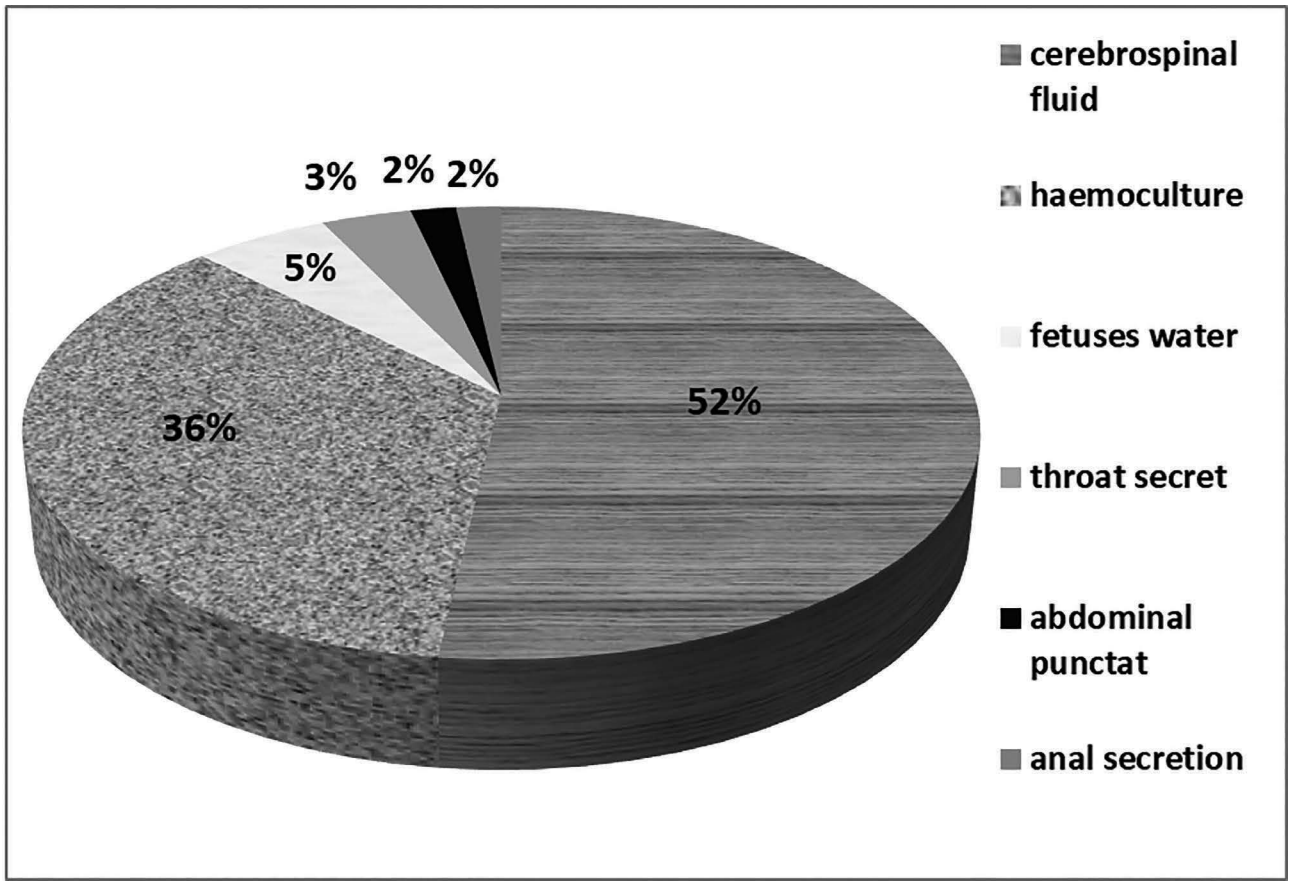

Figure 5. Proportions of clinical samples investigated for Listeria.

The distribution of the studied Listeria strains by hospitals for the studied period is presented in Fig.6. Four serogroups of Listeria were found in "St. George University Hospital", Plovdiv. *Listeria monocytogenes I serogroup* was detected in 6 hospitals and serotype *4b* - in five hospitals. At the same time, both serogroups were found in three hospitals.

The highest percentage of isolated strains was from newborns - 12/56 (21.43%), followed by those isolated from patients aged 60-69 years - 11/56 (19.64%). The fewest strains were isolated from patients in the 10-19 and 40-49 age groups (Fig. 7).

DISCUSSION

It is essential to prepare a spatial epidemiological study of epidemiological features of an infection. In Bulgaria he morbidity of listeriosis during the period 1990-2000was 0.02%$_{000}$ and during the next decade (2001-2010) there were 2 peaks of morbidity (in 2002 - 0.08%000 and in 2007 - 0.14% $_{000}$) (25). During the study period, the incidence of listeriosis was dynamic, with 4 peaks. This is most likely due to more frequent outbreaks as well as the influence of more factors on the epidemic process.

Listeriosis is one of the infectious diseases that often end in death. Despite of the better detection of listeriosis, the persistently high mortality and lethality remain an issue even in recent years. *L. monocytogenes* is the leading cause of high mortality (from 20% to 30%) from foodborne infections in humans (22). In the current study, mortality was higher in three of the years (2012, 2014, 2017). In our country only in 2019 no deaths were reported. Most patients died in 2014 (6 people), when the highest mortality rate was registered - 0.08 % $_{000}$ (25).

Assays (serological and PCR) grouped *Listeria monocytogenes* into 13 serotypes (*1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e* and *7*) (9) with different virulence potential. The studies (18, 19, 23) indicate that most (98%) cases of listeriosis in humans are caused by serotypes *4b, 1/2a, 1/2b* and *1/2c,* and for the most recent sporadic or epidemic cases of listeriosis was responsible serotype *4b*. Different Listeria serotypes are specific to particular geographic areas. Serotype *1/2a* is common in some European countries: Italy, Czech Republic and Poland (4, 11). For our country the predominant serotype is also *1/2a* (50%), followed by serotype *4b*. *Listeria welshimeri* was isolated in only 2% of cerebrospinal fluid strains taken from a 30-year-old man diagnosed with meningitis. This serotype was first isolated in human from a fecal sample in France in 1987. Until then, this serogroup had only been isolated from the environment and from rare animal sources (2). *L. innocua* is almost always non-hemolytic, but a few strains have been found to be hemolytic (24). Biochemically, *L. innocua* is very similar to *L. monocytogenes*. In the present study, it was isolated from cerebrospinal fluid of two patients diagnosed with meningitis. The strains were non-hemolytic.

The predominant Listeria serotype isolated from cerebrospinal fluid was *4b,* while from haemoculture it was *1/2a*. The serotype *1/2a* was also isolated from samples of anal secretions, fetus water, throat secretions and abdominal points. *Listeria monocytogenes II serogroup 4b serotype* was responsible for the latest sporadic or epidemic cases of listeriosis, but it is also more virulent. In some countries, such as Sweden and the United States, there is greater variability in serotypes (20,26), while in Algeria, for example, serotype *4b* is isolated mainly from food products (16).

Analyzing the distribution of Listeria serotypes by hospitals, we found that serotype *1/2a* was detected in 12 of the 17 hospitals included in the study (Fig. 6). Serotype *4b* was detected in 11 of the hospitals, both serotypes simultaneously - in 6 of them. The largest variety of strains was found in Plovdiv, UMBAL "Sv. Georgi" (4 types of serotypes), where the largest number of strains -13/56 (23.21%) were studied. Listeria strains isolated from cerebrospinal fluid 12/13 predominated and only one strain was isolated from haemoculture. Serotype *4b* prevailed for this hospital -5/13. In this hospital, 3 strains belonging to *Listeria innocua* and one to *Listeria welshimeri* were also detected.

The strains belonging to serotype *1/2a* (5/11) predominate in hospital "Maichin dom", Sofia. Of the 11 samples tested, 8 were from haemoculture. Only serotype *4b* was confirmed in five of the studied hospitals: MBAL "Burgas" and MBAL "Kustendil" (samples from cerebrospinal fluid) and UMBAL "St. Anna", MBAL "Shumen" and MBAL "Pazardzhik" (samples from haemoculture).

Only serotype *1/2a* was found in six of the studied hospitals: Varna SBAGAL and Sofia MVR (samples of

cerebrospinal fluid), UMBAL "Aleksandrovska", Vtora MBAL, Sofia and MBAL "Stara Zagora" (samples from haemoculture) and SBALXZ Sofia (sample from abdominal puncture) (Fig. 6). These data confirm a steady trend in the spread of certain listeria serotypes in each hospital over the years. Serotypes' circulation should be considered when analyzing epidemiological data on listeriosis in these regions.

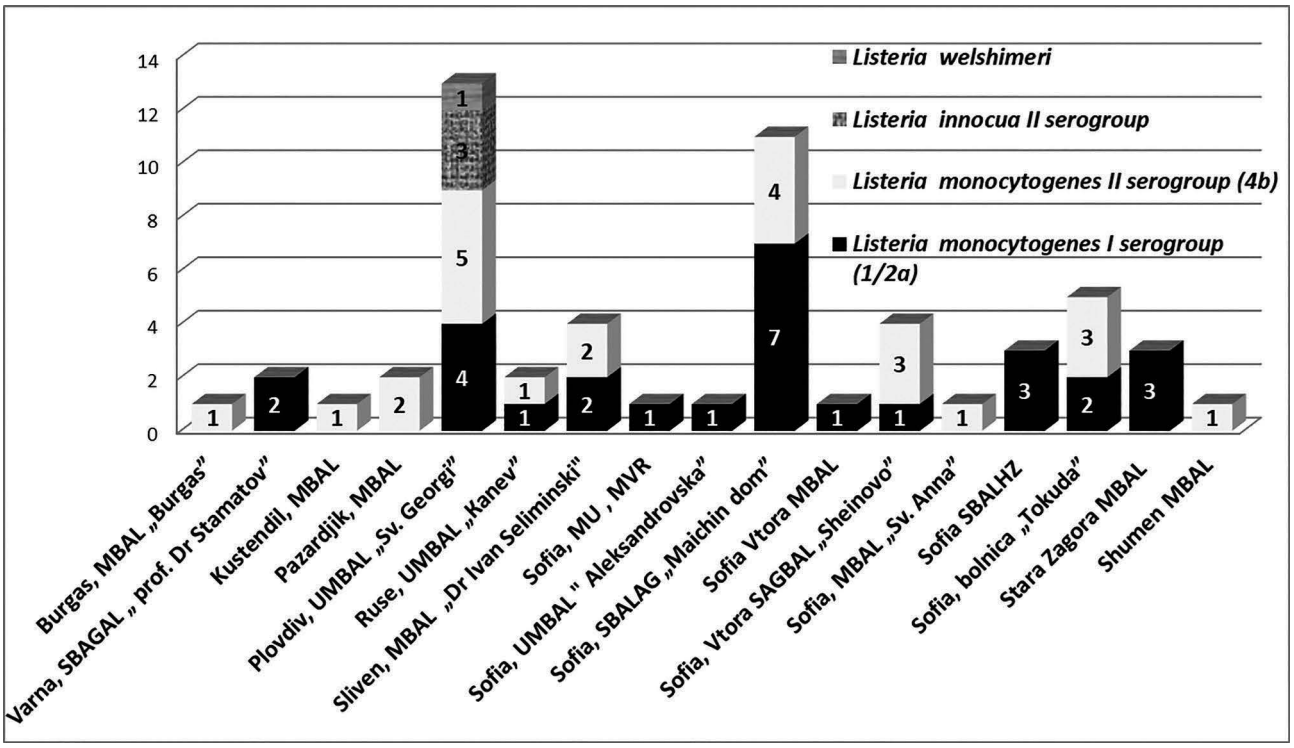

Figure 6. Distribution of Listeria strains by hospitals, 2010-2019.

The high percentage (21.43%) of isolated *Listeria* strains from newborns confirms the fact that they are a major risk group for Listeria infection (Fig.7).

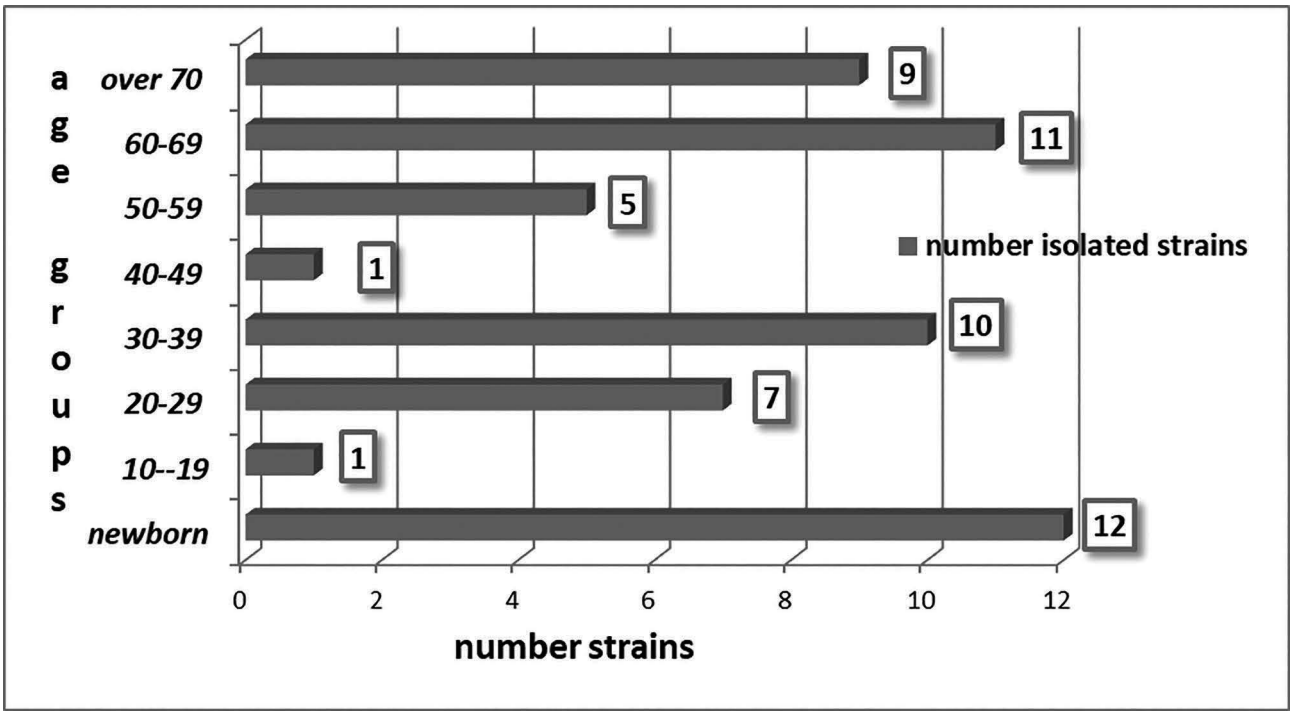

Figure 7. Distribution of Listeria strains by age groups, 2010-2019.

It is known that the risk of listeriosis is highest in vulnerable groups: pregnant women and their embryos, newborns, and immunosuppressed people with chronic liver disease, diabetes, cancer, AIDS or transplants, as well as people in old age (over 65 years). There is evidence lot of literature data for development of severe complications (abortions, premature birth, meningitis, meningoencephalitis, sepsis). They are observed both in animals and humans.Mainly risk groups such as pregnant women, newborns and immunocompromised individuals are affected (3,10).

CONCLUSIONS

Our study emphasizes the need to unite the efforts of microbiologists, immunologists, infectious disease specialists in search of appropriate tests for diagnosis of listeriosis. The emphasis should be on the development and implementation of risk groups screening, especially of women aged 20-40 years with evidence of miscarriage and stillbirth. It is necessary to collect, summarize and analyze information about feeding women of childbearing potential to determine the risk of infection with listeriosis through food.

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