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A survey of laboratory-confirmed isolates of invasive listeriosis in Israel, 1997–2007

V. VASILEV[1]*, R. JAPHETH[1], N. ANDORN[1], R. YSHAI[1], V. AGMON[1], E. GAZIT[2], Y. KASHI[3] AND D. COHEN[4]

[1] *Central Laboratories of the Ministry of Health, Jerusalem, Israel*
[2] *Department of Laboratories of the Ministry of Health, Jerusalem, Israel*
[3] *Faculty of Biotechnology and Food Engineering, Technion – Israel Institute of Technology, Haifa, Israel*
[4] *Department of Epidemiology and Preventive Medicine, School of Public Health, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel*

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SUMMARY

During the 11-year period from 1997 to 2007, 321 isolates of *Listeria monocytogenes* from sporadic cases of invasive listeriosis were reported to the national reference laboratory in Israel. Of these isolates, 113 (35%) were identified from perinatal cases, and 208 (65%) from non-perinatal cases. The prevalent serovars were 4b, 1/2b, 1/2a and 4c. Serovar 4b was identified in 80·5% of the perinatal isolates ($P=0{\cdot}0162$), while the number of 1/2b and 1/2a strains increased in the $\geqslant$60 years old group ($P=0{\cdot}0285$). Resistance to tetracycline was found in eight 4b isolates. The seasonal distribution showed that 206 isolates (64·2%) were submitted during the hot season (May–October). The estimated morbidity for the study period was 4·4 per million. The incidence of invasive listeriosis was higher in the perinatal group (5·6/100 000), than in individuals aged $\geqslant$60 years (1·5/100 000).

Key words: Incidence, listeriosis, resistance, serovars, surveillance.

INTRODUCTION

With a morbidity of 2–10 cases per million, invasive listeriosis is a rare disease; however, it may be a serious health risk for immunocompromised individuals and the elderly because *Listeria monocytogenes* is widely spread and may enter the food chain despite standard precautions [1–3]. The reported figures depend on the sensitivity of surveillance, with the highest rates registered in countries with statutory notification of *Listeria* infections [4, 5]. An integral part of the monitoring system is the microbiological survey, conducted by a central reference laboratory, using phenotypic (serology) and genotypic (PFGE, MLVA and MLST) methods [6, 7]. Since most isolates belong to serovars 4b, 1/2b, and 1/2a, the discriminatory power of *Listeria* serology is relatively low. However, the genetic division of the serovars into lineages and phylogenetic groups has increased the usefulness of serotyping as a relatively quick, low-cost method for routine microbiological surveillance [8].

In Israel, invasive listeriosis has been reported in immunocompromised patients and pregnant women [9–12]. Notification became mandatory in 1996, and the Reference Listeria Laboratory (RLL) was established in 1997. The aim of this report is to present the

* Author for correspondence: Dr V. Vasilev, Central Laboratories of the Ministry of Health, 9 Yaakov Eliav Street, PO Box 34410, Jerusalem 91342, Israel.
(Email: valentine.vasilev@eliav.health.gov.il)

Table 1. *Serovar distribution of* L. monocytogenes *isolates in the host groups*

Serovar	4b	1/2b	1/2a	4c	Total
Perinatal isolates ($P=0{\cdot}0162$)	91 (80·5%)	18 (16%)	4 (3·5%)	0	113 (100%)
Non-perinatal isolates age $\geqslant 60$ years ($P=0{\cdot}0285$)	80 (55·5%)	45 (31%)	17 (12%)	2 (1·4%)	144 (100%)
Non-perinatal isolates age <60 years	44 (69%)	13 (20%)	7 (11%)	0	64 (100%)
All serovars Number (%)	215 (67%)	76 (24%)	28 (8·7%)	2 (0·6%)	321 (100%)

first longitudinal survey of clinical *L. monocytogenes* isolates, tested at the RLL.

MATERIALS AND METHODS

The study includes a total of 321 *L. monocytogenes* isolates from sporadic cases, identified by hospital laboratories, using standard methods [13] over a period of 11 years (1997–2007). Using the data in the accompanying forms, they were divided into perinatal and non-perinatal, and the second group was subdivided into strains from patients aged <60 years and $\geqslant 60$ years (Table 1). The isolates were from blood, CSF, maternofetal (amniotic fluid, placenta and products of conception) and focal sites (pleural and peritoneal fluid).

Biochemical verification was carried out with the Microgen Listeria-ID system (Microgen Bioproducts Ltd, Camberley, Surrey, UK). Serotyping was performed with commercial antisera, following the manufacturer's protocol (Denka Seiken, Tokyo, Japan), with a modification for the H-antigen agglutination test [14].

Antimicrobial susceptibility was checked by the disk diffusion method in accordance with the recommendations of the Swedish Reference Group for Antibiotics [15]. Commercially prepared disks (Oxoid, Basingstoke, UK) were used, containing ampicillin (AMP, 10 μg), sulphamethoxazole–trimethoprim 19.1 (SXT, 25 μg), vancomycin (VA, 30 μg), tetracycline (TE, 30 μg), gentamicin (CN, 10 μg) and chloramphenicol (C, 30 μg). The results were interpreted using the criteria of the National Committee for Clinical Laboratory Standards for *Listeria* spp. and *Staphylococcus* spp., as well as previously published data [16–18]. Reference strains of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were tested in each run.

Data analysis

Correlations between the general prevalence of the serovars and their distributions in the host groups were analysed by χ^2 test (two-tailed tests were used). The morbidity estimates (per million) were calculated using the population figures of the Israel Bureau of Statistics [Statistical abstracts (http://www.cbs.dov.il)]. The incidence estimates (per 100 000) were based on the number of pregnancies and individuals aged <60 and $\geqslant 60$ years.

RESULTS

Perinatal isolates

A total of 113 (35%) isolates were from perinatal cases: 29 newborns and 84 pregnant women (mean age 29 years, range 19–40). The most prevalent serovar was 4b (80·5%), followed by 1/2b and 1/2a ($P=0{\cdot}0162$, Table 1). The isolates were from blood (77, 68%), maternofetal sites (29, 26%) and CSF (7, 6·2%).

Non-perinatal isolates

Of the 208 (65%) non-perinatal isolates, 144 were from patients aged $\geqslant 60$ years (mean age 79 years, range 60–91) and 64 from patients aged <60 years (mean age 42 years, range 2–59). The most prevalent serovar was 4b, followed by significantly high numbers of 1/2b and 1/2a strains in the $\geqslant 60$ years subgroup in comparison with the general serovar

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distribution ($P=0{\cdot}0285$, Table 1). Two 4c strains were identified in blood and CSF from female patients aged 80 and 81 years, respectively. Most of the samples were from blood (134, 78%), followed by CSF (29, 17%) and focal sites (9, 5·2%); 105 patients were male and 103 were female (M/F ratio = 1·01).

The seasonal distribution showed that 206 isolates (64·2%) were submitted between May and October ($P=0{\cdot}0001$).

Antimicrobial susceptibility

A total of 115 *L. monocytogenes* strains were checked, of which 96 (83·5%) were from blood, 13 (11·3%) from CSF, and 6 (5·2%) from maternofetal sites. The serovar distribution was as follows: 4b (80, 69·5%), 1/2b (17, 15%), 1/2a (16, 14%) and 4c (2, 2%). Resistance to TE was found in eight 4b isolates (6·5%), of which seven were from blood and one from CSF. Three of the resistant strains were from perinatal cases and five from non-perinatal cases.

DISCUSSION

In our population 4b was the most common *Listeria* serovar isolated over an 11-year period. This result is consistent with its recognized high pathogenic potential [19]. As previously noted [19, 20], serovar 4b is more common in the perinatal group ($P=0{\cdot}0162$), compared with those aged $\geqslant 60$ years ($P=0{\cdot}0285$, Table 1). The different distribution of serovar 1/2b in the two groups may be connected to genetic variances in pathogenic tropism, since it is of the same lineage as 4b but in a separate phylogenetic group [8].

The prevalence of 1/2a, which belongs to a different lineage (lineage II), differs from other reports where it has been the most common or the second most common serovar in clinical strains [1, 21]. Its occurrence may also be host dependent, in that it is less commonly isolated from perinatal cases, and increases in those aged $\geqslant 60$ years (Table 1).

Serovar 4c (lineage III) is rarely found in clinical isolates [22, 23]. In this study, both strains were rhamnose +, which places them in the genetically distinct and reportedly more virulent subgroup IIIA [22]. However, low exposure to food contaminated with *Listeria* isolates belonging to the three subgroups of serovar 4c may be a reason for the small number of clinical cases described in our population [22, 24].

The noted summer increase in morbidity [1, 12, 25] was also found in our study, with the May–October period being the hot season in Israel. According to one hypothesis, there is a link with the concomitant increase in gastrointestinal infections, which, in addition to provoking immunosupression, facilitate the entry and dissemination of *L. monocytogenes* in the intestine [2, 26, 27]. There is a well-documented case report of sporadic listeriosis in a healthy adult following *Shigella sonnei* shigellosis [28], as well as experimental data of interactions between *L. monocytogenes* and other enteric bacteria at the level of the intestinal mucosa [29]. The relevance of these findings to listeriosis incidence requires additional studies, considering the established high morbidity of shigellosis and other enteric diseases in Israel [30].

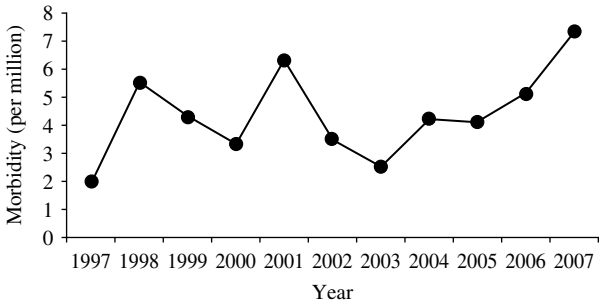

Fig. 1. Estimated annual morbidity (per one million).

As in other reports, antimicrobial susceptibility is high, with tetracycline resistance most often described in human isolates when antimicrobial resistance is present [31]. Incidentally, a low incidence of tetracycline resistance also was found in food isolates in Israel from the same period (V. Vasilev, unpublished data).

Based on the number of submitted isolates, the morbidity of invasive listeriosis in Israel is 4·4 per million. Since 2005, the number of reported *Listeria* cases has been increasing (Fig. 1), while most industrialized countries report a decline [32]. The increase in *Listeria* cases in Israel requires further study to understand its aetiology. As previously reported by others [1, 4, 19, 33], invasive listeriosis was more common in the perinatal age group (5·6/100 000) than in individuals aged $\geqslant 60$ years (1·5/100 000).

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DECLARATION OF INTEREST

None.

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