Polish Journal of Microbiology
2004, Vol. 53, No 4, 279–281

Antimicrobial Susceptibilities of *Listeria monocytogenes* Strains Isolated from 2000 to 2002 in Poland

JAROSŁAW PACIOREK

National Institute of Hygiene, Warsaw, Poland

Received 27 July 2004

A b s t r a c t

Minimal inhibitory concentration (MIC) of ampicillin, penicillin, gentamicin, erythromycin, clarithromycin, sulfisoxazole and trimethoprim for 73 *Listeria monocytogenes* isolates from clinical material samples, food and environment was determined using broth microdilution method. With exception of the sulfonamide all antibacterial agents were active against all tested strains. Resistance to sulfisoxazole (MIC ≥ 512 μg/ml) was detected in case of 30.1% of isolates including 5 of 14 human strains.

K e y w o r d s: *Listeria monocytogenes*, MIC, antimicrobial susceptibility

Listeria monocytogenes is a causative agent of listeriosis, which can occur sporadically or epidemically. In most cases listeriosis has a food-borne origin (Kathariou, 2002). Neonates, pregnant women, the elderly, immunosuppressed transplant recipients and others with impaired cell-mediated immunity belong to a group with the greatest risk of development of listeriosis. Most frequent forms of listeriosis include meningoencephalitis, bacteremia and perinatal infections (Doganay, 2003). Although morbidity is relatively low (2–10 reported cases per million population per year) listeriosis is characterised by a high mortality rate – 20–30%. Therefore listeriosis remains a great public health concern (de Valk *et al.*, 2003).

Although *L. monocytogenes* is susceptible *in vitro* to a wide range of antimicrobial agents, in practice – due to the ability of the bacterium to penetrate the central nervous system and to reside and multiply intracellularly – only few antimicrobials are routinely used in therapy of listeriosis. Ampicillin, amoxicillin or penicillin combined with gentamicin remain the therapy of primary choice. For patients allergic to β-lactams, the combination of trimethoprim with a sulfonamide such as sulfamethoxazole, as in co-trimoxazole, has been recommended as a therapy of second choice. In case of bacteraemic listeriosis during pregnancy, erythromycin monotherapy has been successful (Hof, 2004; Jones and MacGowan, 1995; Temple and Nahata, 2000).

Although the majority of *L. monocytogenes* isolates appear susceptible to antibiotics and chemotherapeutics used in therapy of listeriosis, an increasing number of reports describing resistance have been found, including strains resistant to ampicillin, penicillin, gentamicin, erythromycin, sulfonamide and trimethoprim (reviewed in Poroś-Głuchowska and Markiewicz, 2003). Such data suggest the need of monitoring *L. monocytogenes* for the presence of resistant strains.

The aim of this study was to gain insight into the incidence of resistance to certain antimicrobials by *L. monocytogenes* strains isolated in Poland during 2000–2002.

L. monocytogenes isolates (73) from clinical material samples (14), food (50), environment (8) and a diseased goat (1) were received from 4 laboratories in Warsaw and from laboratories in Szczecin, Puławy, Gdańsk and Bydgoszcz. Strains were re-identified at the National Institute of Hygiene by Api Listeria system (BioMérieux). *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used as control organisms.

Correspondence address: National Institute of Hygiene, Dept. of Bacteriology, 24 Chocimska St., 00-791 Warsaw, Poland, e-mail: j.paciorek@pzh.gov.pl

The following antimicrobial agents were used: ampicillin, penicillin G, gentamicin, erythromycin, sulfisoxazole, trimethoprim (Sigma) and clarithromycin (Abbott Laboratories). They were dissolved and diluted according to manufacturers instructions and Hindler (1992).

Minimal inhibitory concentration of antibacterial agents (MIC) was determined by the microdilution broth method according to National Committee for Clinical Laboratory Standards (NCCLS, 2003) and Hindler (1992). Briefly, serial two-fold dilutions of the agents were prepared in the U-bottom 96-well sterile microtiter plates. The drugs were diluted with Mueller-Hinton broth (Oxoid) supplemented with Ca^{2+}, Mg^{2+}, and 2.5% of final concentration of lysed horse blood (LHB-CAMH). For assays with trimethoprim and sulfisoxazole, thymidine phosphorylase (Sigma) was added to convert the thymidine pool to thymine (final concentration – 0.2 IU/ml). Overnight cultures on BHI agar plates were adjusted to a 0.5 McFarland standard using sterile NaCl (0.9%) and then properly diluted in LHB-CAMH. The inoculum (50 µl of approximately 5×10^4 colony forming units) was added to each well in microtiter plates containing 50 µl of an antimicrobial agent dilution. Plates were incubated for 18-h at 35ºC. For each strain a growth control well, a purity plate and an inoculum count verification plate were also inoculated. For each microtiter plate uninoculated broth sterility control was included. MIC was defined as the lowest concentration of antibacterial agent that prevented macroscopically visible growth or, for sulfisoxazole, that inhibited *c.a.* 80% of growth compared with that in the growth control well after 18 h of incubation. MIC_{50} and MIC_{90} include 50 and 90%, respectively, of strains with the given or lower MIC value.

Breakpoints were those recommended by NCCLS (2002) for *Staphylococcus* spp. except for ampicillin and penicillin where specific *Listeria* breakpoints are defined (see Table I).

Table I
Minimal inhibitory concentrations (MIC) of the tested antibacterial agents for *L. monocytogenes* isolated in Poland between 2000–2002

Antimicrobial agent	MIC (µg/ml)			NCCLS criteria (µg/ml)*		
	Range	50%	90%	S	I	R
Ampicillin	≤0.06–0.5	0.25	0.25	≤2		
Penicillin	0.03–0.5	0.25	0.25	≤2		
Gentamicin	0.03–0.5	0.06	0.12	≤4	8	≥16
Erythromycin	0.06–0.25	0.12	0.25	≤0.5	1–4	≥8
Clarithromycin	≤0.03–0.12	0.06	0.12	≤2	4	≥8
Trimethoprim	≤0.03–0.5	≤0.03	≤0.03	≤8	–	≥16
Sulfisoxazole	2–>512	64	>512	≤256	–	≥512

S – susceptible, I – intermediate, R – resistant
* for details see text

The ranges, MIC_{50} and MIC_{90} of the seven antimicrobial agents tested for 73 *L. monocytogenes* isolates are shown in Table I. With the exception of sulfisoxazole all strains were susceptible to all drugs tested. Trimethoprim had the lowest MIC for 90% of the strains (≤0.03 µg/ml). It is worth mention that successful trimethoprim monotherapy of meningitis following co-trimoxazole has been described (Gunther and Philipson, 1988) and that *Listeria* resistance to trimethoprim is very rare (Hof *et al.*, 1997). There were no differences in the activity of both β-lactams tested. As for the macrolides, clarithromycin was slightly more active than erythromycin, which is in agreement with some earlier reports (Hof *et al.*, 1997). Resistance to sulfisoxazole (MIC ≥512 µg/ml) was detected in 22 strains (30.1%) including 5 of 14 human isolates. Conflicting results regarding activity of sulfonamides against *Listeria* isolates have been published. Some authors reported susceptibility of the strains tested (Hof *et al.*, 1997; Larsson *et al.*, 1985; Winslow and Pankey, 1982), while others described resistance of many isolates to the sulfonamide (Franco Abuin *et al.*, 1994). It was even suggested that *Listeria* spp. is naturally resistant to sulfamethoxazole (Troxler *et al.*, 2000). This confusion might reflect the lack of a standardised testing procedure – a variety of methods (broth microdilution, agar dilution, disk diffusion), media (Mueller-Hinton, CAMH, TSB) or even incubation conditions (*e.g.* 35°C or 37°C) were used. It was shown *e.g.* that 76% of isolates susceptible to sulfisoxazole according to broth microdilution test had been classified as resistant due to disk diffusion method

results (Vela *et al.*, 2001). Other potential reasons for the discrepancies mentioned above might remain obscure unless one standardised test protocol is implemented.

Acknowledgements. The author is grateful to Maja Ławrynowicz-Paciorek for advice and help in preparation of microtiter plates, and to Aleksandra A. Zasada and Ewa Sumińska for their help in MIC determination.

Literature

Doganay M. 2003. Listeriosis: clinical presentation. *FEMS Immunol. Med. Microbiol.* **35**: 173–175.

Franco Abuin C.M., E.J. Quinto Fernández, C. Fente Sampayo, J.L. Rodriguez Otero, L. Dominguez Rodriguez and A. Cepeda Sáez. 1994. Susceptibilities of *Listeria* species isolated from food to nine antimicrobial agents. *Antimicrob. Agents Chemother.* **38**: 1655–1657.

Gunther G. and A. Philipson. 1988. Oral trimethoprim as follow-up of meningitis. *Rev. Infect. Dis.* **10**: 53–55.

Hindler J. 1992. Antimicrobial Susceptibility Testing, p. 5.1.1.–5.25. In: H.D. Isenberg (ed.), Clinical Microbiology Procedures Handbook, American Society for Microbiology, Washington, D.C.

Hof H. 2004. An update on medical management of listeriosis. *Expert. Opin. Pharmacother.* **5**: 1727–1735.

Hof H., T. Nichterlein and M. Kretschmar. 1997. Management of listeriosis. *Clin. Microbiol. Rev.* **10**: 345–357.

Jones E.M. and A.P. MacGowan. 1995. Antimicrobial chemotherapy of human infection due to *Listeria monocytogenes*. *Eur. J. Clin. Microbiol. Infect. Dis.* **14**: 165–175.

Kathariou S. 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *J. Food Prot.* **65**: 1811–1829.

Larsson S., M.H. Walder, S.G. Cronberg, A.B. Forsgren and T. Moestrup. 1985. Antimicrobial susceptibilities of *Listeria monocytogenes* strains isolated from 1958 to 1982 in Sweden. *Antimicrob. Agents Chemother.* **28**: 12–14.

NCCLS. 2002. Performance standards for antimicrobial susceptibility testing; twelth informational supplement. NCCLS document M100-S12, NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania.

NCCLS. 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard – sixth edition. NCCLS document M7-A6, NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania.

Poroś-Głuchowska J. and Z. Markiewicz. 2003. Antimicrobial resistance of *Listeria monocytogenes*. *Acta. Microbiol. Pol.* **52**: 113–129.

Temple M.E. and M.C. Nahata. 2000. Treatment of listeriosis. *Ann. Pharmacother.* **34**: 656–661.

Troxler R., A. von Graevenitz, G. Funke, B. Wiedemann and I. Stock. 2000. Natural antibiotic susceptibility of *Listeria* species: *L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri* and *L. welshimeri* strains. *Clin. Microbiol. Infect.* **6**: 525–535.

de Valk H., Ch. Jacquet, V. Goulet, V. Vaillant, A. Perra, J-C. Desenclos, P. Martin and the Listeria Working Group. 2003. Feasibility study for a collaborative surveillance of *Listeria* infections in Europe. Report to the European Commission, DGSANCO, Paris.

Vela A.I., J.F. Fernández-Garayzábal, M.V. Latre, A.A. Rodriguez, L. Dominiguez and M.A. Moreno. 2001. Antimicrobial susceptibility of *Listeria monocytogenes* isolated from meningoencephalitis in sheep. *Int. J. Antimicrob. Agents.* **17**: 215–220.

Winslow D.L. and G.A. Pankey. 1982. In vitro activities of trimethoprim and sulfamethoxazole against *Listeria monocytogenes*. *Antimicrob. Agents Chemother.* **22**: 51–54.