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

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Abstract

Minimal inhibitory concentration (MIC) of ampicillin, penicillin, gentamicin, erythromycin, clariromycin, 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.

Key words: *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 Poros- Gluchowska 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.

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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<sup>2+</sup>, Mg<sup>2+</sup>, 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<sup>4</sup> colony forming units) was added to each well in microtiter plates containing 50 μl of an antimicrobial agent dilution. Plates were incubated for 18-24h at 35°C. 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 <i>c.a. 80</i>% of growth compared with that in the growth control well after 18 h of incubation. MIC<sub>50</sub> and MIC<sub>90</sub> include 50 and 90%, respectively, of strains with the given or lower MIC value.

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

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (μg/ml)</th>
<th>NCCLS criteria (μg/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>≤0.06–0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.03–0.5</td>
<td>0.25</td>
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<tr>
<td>Gentamicin</td>
<td>0.03–0.5</td>
<td>0.06</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.06–0.25</td>
<td>0.12</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>≤0.03–0.12</td>
<td>0.06</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>≤0.03–0.5</td>
<td>≤0.03</td>
</tr>
<tr>
<td>Sulfisoxazole</td>
<td>2–&gt;512</td>
<td>64</td>
</tr>
</tbody>
</table>

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

The ranges, MIC<sub>50</sub> and MIC<sub>90</sub> of the seven antimicrobial agents tested for 73 <i>L. monocytogenes</i> 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 <i>Listeria</i> 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 sulphonamides against <i>Listeria</i> 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 sulphonamide (Franco Abuin et al., 1994). It was even suggested that <i>Listeria</i> 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.

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Literature

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