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Antibiotic susceptibility of 259 *Listeria monocytogenes* strains isolated from food, food-processing plants and human samples in Germany

Matthias Noll^{a,b,*}, Sylvia Kleta^b, Sascha Al Dahouk^{b,c}

^a University of Applied Sciences and Arts, Institute for Bioanalysis, Friedrich-Streib-Str. 2, D-96450 Coburg, Germany

^b German Federal Institute for Risk Assessment, National Reference Laboratory for *Listeria monocytogenes*, Diederichsdorfer Weg 1, D-12277 Berlin, Germany

^c RWTH Aachen University, Department of Internal Medicine III, Pauwelsstraße 30, D-52074 Aachen, Germany

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ABSTRACT

Background: The objective of this study was to evaluate the susceptibility of 259 *Listeria monocytogenes* strains isolated from food and food-processing environments and patient samples in Germany to 14 antibiotics widely used in veterinary and human medicine. *L. monocytogenes* strains were isolated mainly from milk and milk products and classified according to their molecular serotypes IIa (n = 112), IIb (n = 41), IIc (n = 36), IVa (n = 1), IVb (n = 66), and IVb-v1 (n = 3).

Methods: Susceptibility tests were performed by using the automated 96-well based microdilution system Micronaut-S. Ampicillin, benzylpenicillin, ceftriaxone, ciprofloxacin, daptomycin, erythromycin, gentamicin, linezolid, meropenem, rifampicin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole and vancomycin were tested in at least five different concentrations.

Results: Among the 259 strains under study, 145 strains revealed multidrug-resistance (resistance to ≥ 3 antibiotics) and predominantly belonged to serotype IV (59%). Strains were mainly resistant to daptomycin, tigecycline, tetracycline, ciprofloxacin, ceftriaxone, trimethoprim/sulfamethoxazole and gentamicin.

Conclusions: Antibiotic resistance in general and multidrug-resistance in particular were more prevalent in *L. monocytogenes* strains isolated in Germany compared to similar reference stocks from other European countries and the USA but similar to stocks from China.

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Introduction

Listeria monocytogenes is a Gram-positive pathogenic bacterium that can cause listeriosis, a severe invasive infection in humans. These organisms are among the most important causes of death from foodborne infections in industrialized countries with an average case fatality rate of 17.7% in the European Union [1]. Listeriosis is of public health concern all over the world, with an increasing incidence in Europe, especially among the elderly [2–4]. The EU notification rate was 0.44 per 100 000 population in 2015, which corresponds to an increase of 8.6% compared with 2012 [1]. However, country-specific notification rates were quite variable ranging from 0.04 (Bulgaria) to 1.12 (Finland) per 100 000 population [1]. Although optimal therapy of listeriosis has not been verified by

randomized clinical trials, penicillin or ampicillin alone, or in combination with gentamicin are considered to be the drugs of choice [5,6]. The gold standard in the diagnosis of listeriosis is the isolation of the bacteria from normally sterile sites such as blood, cerebrospinal fluid, amniotic fluid, and placenta. To isolate *L. monocytogenes* from samples of non-sterile sites such as gastric lavages, oral and ear swabs or environmental samples, selective enrichment is required for successful culture [7]. *L. monocytogenes* strains are primarily differentiated either by conventional or molecular serotyping, and the majority of clinical isolates belongs to the molecular serotypes IVb, IIa and IIc [8].

The consumption of contaminated food products is the main route of transmission [9,10], which can be mainly observed for ready-to-eat food such as deli meat, raw milk products, sausages or smoked fish [8,11]. *L. monocytogenes* is ubiquitous in nature and many strains are able to survive a broad spectrum of harsh conditions such as acidic environment [12], low temperatures [13] and high salt concentrations [14].

* Corresponding author at: University of Applied Science and Arts, Institute for Bioanalysis, Friedrich-Streib-Str. 2, D-96450 Coburg, Germany.

E-mail addresses: matthias.noll@hs-coburg.de (M. Noll), Sylvia.Kleta@bfr.bund.de (S. Kleta), Sascha.Al-Dahouk@gmx.de (S. Al Dahouk).

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Table 1
Sources and molecular serotypes of the *L. monocytogenes* field strains (n = 259) under study.

Source	IIa	IIb	IIc	IVa	IVb	IVb-v1	Total
Crustaceans and molluscs	2	0	0	0	1	0	3
Fish and fish products	19	5	4	0	12	2	42
Food production environment	6	5	5	0	5	1	22
Human	6	5	3	0	8	0	22
Meat and meat products	19	14	18	0	20	0	71
Milk, cheese and other dairy products	57	9	5	1	16	0	88
Vegetarian food	3	3	1	0	4	0	11
Total	112	41	36	1	66	3	259

With the exception of natural *in vitro* resistance to first generation quinolones, fosfomycin, and third-generation cephalosporin, *L. monocytogenes* is widely susceptible to clinically-relevant classes of antibiotics active against Gram-positive bacteria [6,15–20].

To the best of our knowledge, in 1988 the first multidrug-resistant *L. monocytogenes* strain described was isolated from a patient in France suffering from meningoencephalitis [21]. Since then, various strains of *L. monocytogenes* have been isolated from food, environmental or clinical samples originating from sporadic human and animal listeriosis cases which showed resistance to several antibiotics [11,22,23]. Antibiotic susceptibility testing of *Listeria* was hitherto performed by disc diffusion using Mueller–Hinton agar [15,17–20,24], or by broth microdilution methods [6,16,25,26]. Although only a few globally distributed clones could have been identified to date [27], antibiotic susceptibility of *L. monocytogenes* strains varied widely depending on sampling sites and geographic origin.

The main objective of this study was to evaluate the susceptibility of 259 *L. monocytogenes* strains isolated from food, food-processing plants and listeriosis patients in Germany to 14 antibiotics frequently used in veterinary and human medicine.

Material and methods

Strains

The 259 *L. monocytogenes* strains included in this study have been collected over a period of 40 years. Each strain was isolated from an individual source comprising raw and pasteurized milk, meat, fish, food products, food processing plant environments and human patients in Germany (Tables 1 and S1). All strains were identified by biochemical and molecular methods according to ISO 11290-1:1996+AMD 1:2004 and Bubert and colleagues [28]. In addition, molecular serotyping was conducted [29,30]. Finally, all strains were archived in the strain collection of the National Reference Laboratory for *L. monocytogenes* located at the German Federal Institute for Risk Assessment in Berlin where they were stored at -80°C until use. *L. monocytogenes* strains were selected by serotypes most frequently associated with human listeriosis cases, i.e. IIa, IIb and IVb [5] (Table 1).

These strains mainly originated from milk, cheese and other dairy products. Furthermore, 35 reference strains of the genus *Listeria* provided by various strain collections were included in the study (Table 2).

Antibiotic susceptibility testing

L. monocytogenes strains were plated on sheep blood agar (Mast Diagnostika DM 101, Reinfeld, Germany) and incubated for 24 h at 37°C . Antibiotic susceptibility testing was conducted according to manufacturer's guidelines (Merlin Diagnostika, Bornheim, Germany). Briefly, one colony of each *L. monocytogenes* strain under study was transferred into 2 mL of a 0.9% NaCl solution, and turbidity was adjusted to a 0.5 McFarland standard (VWR International,

Ismaning, Germany). A total of 200 μL of the bacterial solution were admixed to 11 mL of H-broth (Merlin Diagnostika). Finally, 100 μL of this suspension were added to each well of a 96-well Micronaut[®] microtiter plate (Merlin Diagnostika). The plate was gently shaken for 1 min to resolve the lyophilized drugs and to mingle antimicrobial agents with the bacteria, and thereafter incubated for 24 h at 37°C . The setup of antibiotics and their concentrations used in the Micronaut[®] microtiter plate are shown in Fig. 1.

After incubation, the optical density of each well was measured at a wavelength of 690 nm (MRX[®], Dynex Technologies Inc., Chantilly, VA, USA). Bacterial growth related to antibiotic resistance was proven by an $\text{OD}_{690} > 0.13$ whereas sensitive *L. monocytogenes* strains showed an $\text{OD}_{690} < 0.13$, given that the controls including the same *L. monocytogenes* strain without antibiotics and H-broth without bacteria revealed an $\text{OD}_{690} > 0.16$ and < 0.16 , respectively. General criteria to interpret antibiotic susceptibility of *L. monocytogenes* are not available, except for ampicillin, benzylpenicillin, erythromycin, meropenem, and trimethoprim/sulfamethoxazole [31]. Therefore, missing breakpoints were complemented by those recommended for *Staphylococcus* spp. by the European Committee on Antimicrobial Susceptibility Testing [31] (Table 3). We used *Streptococcus pneumoniae* (ATCC49619) as a control for the antibiotic susceptibility tests. Descriptive statistical analyses were carried out with the software Excel of the Professional Plus 2013 package (Microsoft, Redmond, WA, USA). To test a temporal increase in resistance patterns, *L. monocytogenes* strains isolated from milk, cheese and other milk products in 1994 and 2009/2010 were compared (see Table S1 for details). For this purpose, all isolates were randomly selected by Mersenne twister algorithm with an equal distribution and choice without returning [32]. The proportions of resistant strains and non-resistant strains were compared using the chi-square test.

Results and discussions

Antibiotic resistance patterns

The number of *L. monocytogenes* strains resistant or susceptible to 14 different antibiotics frequently used in human and veterinary medicine are presented in Table 3.

All tested strains were resistant against daptomycin, which is why this drug cannot be recommended for antibiotic treatment of listeriosis patients [26]. The frequency of tetracycline resistance (22.8%) and the rare occurrence of erythromycin resistance (1.9%) were in accordance with previous studies [33–37]. Cephalosporin antibiotics (such as ceftriaxone) have been rarely tested in surveys on the susceptibility of *L. monocytogenes* but Davis and Jackson [6] found a high prevalence of resistant strains similar to our study. About 38% of the *L. monocytogenes* strains in our study were resistant towards tigecycline, which is a drug of last resort (DoLR). In contrast, tigecycline revealed excellent *in vitro* activity against *L. monocytogenes* at $\leq 0.5 \mu\text{g}/\text{mL}$ in a previous study [38] and isolates from a slaughterhouse in Romania did not show resistance towards tigecycline at all [39]. Tigecycline resistance may be associated with

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