Natural antibiotic susceptibility of *Listeria* species: *L. grayi, L. innocua, L. ivanovii, L. monocytogenes, L. seeligeri* and *L. welshimeri* strains

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Objective To investigate the natural susceptibility to 71 antimicrobial agents of 103 *Listeria* strains belonging to all known *Listeria* species (*L. monocytogenes* ($N = 21$), *L. innocua* ($N = 21$), *L. seeligeri* ($N = 21$), *L. ivanovii* ($N = 19$), *L. welshimeri* ($N = 11$), and *L. grayi* ($N = 10$)).

Methods MICs were determined using a microdilution procedure in H-Medium.

Results All listeriae were naturally sensitive or intermediate to tetracyclines, aminoglycosides, penicillins (except oxacillin), loracarbef, cefazoline, cefaclor, cefotiam, cefoperazone, carbapenems, macrolides, lincosamides, glycopeptides, dalfopristin/quinupristin, chloramphenicol and rifampicin (probably except *L. grayi*). *Listeria* spp. were naturally resistant or intermediate to most 'modern' cephalosporins (cefetamet, cefixime, ceftibuten, ceftazidime, cefdinir, cefpodoxime, cefotaxime, ceftriaxone, cefuroxime), aztreonam, pipemidic acid, dalfopristin quinupristin and sulfamethoxazole. Significant differences in natural susceptibility among the species were seen with the quinolones, trimethoprim, co-trimoxazole, rifampicin, fosfomycin and fusidic acid. It seems likely that *L. grayi* is naturally resistant to all antifolates; the species was least susceptible to rifampicin and most susceptible to quinolones, whereas *L. ivanovii* was naturally resistant to most quinolones. *L. ivanovii* was naturally sensitive to fosfomycin, whereas *L. innocua* and *L. monocytogenes* were naturally resistant. *L. ivanovii* was also the most susceptible species to fusidic acid.

Conclusions The present study describes a database on the natural susceptibility of *Listeria* spp. to a wide range of antibiotics, which can be used to validate susceptibility testing results of these microorganisms.

Keywords Natural antibiotic susceptibility, *Listeria monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri*, *L. grayi*, MIC, H-Medium, CAMHB, naturally sensitive, naturally resistant

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INTRODUCTION

Within the *Clostridium–Lactobacillus–Bacillus* branch of bacteria, the genus *Listeria* forms a relatively homogeneous taxon of Gram-positive, motile, facultative anaerobic microorganisms which is most closely related to the genus *Brochothrix* [1,2]. Listeriae are widely distributed in nature, having been isolated from the feces of humans and several animals, from different soils, plants, and aquatic environments, and food of animal and vegetable origin (cheese, milk, eggs, sausages, vegetables) [1]. Six *Listeria* species are known to exist in two closely related but distinct lines of descent [3,4]; one line includes the important pathogens *L. monocytogenes* and *L. ivanovii*, but also 'apathogenic' listeriae (*L. innocua*, *L. seeligeri*, and *L. welshimeri*); the other line contains the strains of *L. grayi*. An additional 'species', '*L. murrayi*', was shown to be closely related to *L. grayi* and was reclassified into a subgroup of *L. grayi* in 1992 [5]; *L. grayi* is currently considered to contain two subspecies called *L. grayi* subsp. *grayi* and *L. grayi* subsp. *murrayi*.

From a medical point of view, *L. monocytogenes* is the most important *Listeria* species, causing a wide spectrum of clinical syndromes in humans, summarized as listeriosis. Clinical features range from mild influenza-like illness to meningitis, frequently accompanied by septicemia, and meningoencepha-

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litis [1]. *L. ivanovii* mainly causes abortion in sheep, but cases of listeriosis in cattle and humans have also been reported [6–8]. Although human listeriosis due to listeriae not belonging to *L. monocytogenes* and *L. ivanovii* seems to be exceptional, these species possess the potential to cause human disease. Strains of nearly all *Listeria* species are consumed through food, drinks (including pasteurized milk) and water, often in large amounts, and pathogenic properties have been found in 'apathogenic' listeriae as well [9,10]. Treatment of infections due to opportunistic bacteria may become a problem, because many of these bacteria, although generally showing low virulence, are naturally resistant to numerous antibiotics.

With the exception of *L. monocytogenes*, little information is available on the antimicrobial susceptibilities of *Listeria* spp., including natural susceptibility patterns. The aim of the present study was to establish a database of the natural susceptibilities of all known *Listeria* species to a wide range of antibiotics. In particular, it was investigated whether there are differences in natural susceptibilities between the species.

MATERIALS AND METHODS

Bacterial strains

Twenty *L. innocua*, 20 *L. seeligeri*, 19 *L. monocytogenes*, 18 *L. ivanovii* (subsp. *ivanovii*) and 10 *L. welshimeri* strains were kindly provided by Merlin-Diagnostika (Bornheim, Germany) and Gabi Eifert (Beerfelden, Germany). All these strains were derived from the Special *Listeria* Culture Collection (SLCC) of Heinz Seeliger, actually held by Herbert Hof, Mannheim, Germany. The tested SLCC strains were predominantly isolated in the USA and different European countries (Austria, Belgium, Germany, France, Hungaria, Luxembourg, Norway, Slovakia, Switzerland, UK) and were of clinical, mammalian and environmental origin. *L. innocua*, *L. seeligeri* and *L. welshimeri* strains were mainly isolated from grass, cereals and forage plants, but isolates from animals and human feces were included. The tested SLCC strains of *L. ivanovii* were derived from sheep, rodents, monkeys, human feces, cereals and leaves. Most *L. monocytogenes* strains were of human origin and comprised blood and cerebrospinal fluid specimens, but sheep and avian isolates were also tested. *L. monocytogenes* SLCC 2482 (source not available), *L. monocytogenes* ATCC 15313 (isolated from a rabbit), *L. innocua* ATCC 33090 (isolated from a cow brain) and one food isolate of *L. ivanovii* subsp. *ivanovii* and *L. seeligeri* were kindly provided by Johannes Krämer, Bonn, Germany. Five *L. grayi* strains were kindly submitted by Jacques Bille (Lausanne, Switzerland). Apart from one sheep isolate, these strains originated from humans and were taken from blood ($N = 2$), cerebrospinal fluid and a swab from a nose. *L. grayi* ATCC 19120 (isolated from chincilla feces) was kindly provided by Jocelyn Rocourt (Institut Pasteur, Paris, France). The type strain of '*L. murrayi*', *L. grayi* subsp. *murrayi* ATCC 25401 (isolated from standing corn stalks and leaves in the USA) and *L. grayi* CCUG 24933 (isolated from human feces in Italy) were derived from the Swedish Culture Collection of the University of Göteborg. Two additional *L. grayi* strains of unknown origin and a food isolate of *L. welshimeri* were kindly provided by Herbert Hof, Mannheim, Germany. All strains from clinical specimens were initial isolates from different patients.

Identification

Identification was carried out as described previously [11]. The assignment of *L. ivanovii* strains to *L. ivanovii* subsp. *ivanovii* was performed biochemically on the basis of acid production from ribose and *N*-acetyl-β-D-mannosamine, as described by Boerlin et al [12].

Antibiotic susceptibility testing

MICs were determined using a microdilution procedure in H-Medium, consisting of agarose (4.5 g), Columbia broth (210 g) (Difco Laboratories, Detroit, MI, USA), glucose (30 g), yeast extract (30 g) (Difco Laboratories), neopeptone (12 g) (Difco Laboratories), aqua dest. (4830 mL), hematin solution (540 mL, 0.3 g of hematin added to 600 mL of 0.1M NaOH) and Tween-80, 10% (90 mL). Nine milliliters of pyridoxine–NAD solution (0.2 g of pyridoxine + 0.5 g of NAD + 3000 mL of aqua dest.) and 1 mL of an appropriate bacterial suspension in physiologic saline was added to 90 mL of H-medium, resulting in a mixture containing 3×10^5 CFU/mL. Cultures (18–24 h) of the bacteria grown on sheep blood agar at 36 °C under CO_2 pressure (5%) were used for the preparation of the inocula. Antibiotic-containing microtiter plates (Merlin-Diagnostica, Bornheim, Germany) were inoculated with 100 μL of the mixture and incubated for 20 h at 36 °C. MIC values were read by a photometer (Labsystems Multiskan Multisoft, Helsinki, Finland), and the data were evaluated using a table calculation program. In addition to H-Medium, representative strains of each species were also tested in cation-adjusted Mueller–Hinton broth (CAMHB, Difco Laboratories). For susceptibility testing using this medium, an incubation time of 48–72 h (36 °C) was required.

Evaluation of natural antibiotic susceptibility

Plotting the MIC of a particular antibiotic for one species against the number of strains found with the respective MIC usually results in a bimodal distribution. One peak with relatively low MICs represents the natural population, and one peak with higher MICs represents the strains with acquired (secondary) resistance. Analysis of the MIC distribution of all

strains of one species for each antibiotic permitted determination of the biological thresholds, which limit the natural population at high MICs but not those strains with secondary resistance. Whether the MIC values of the natural population were above or below the breakpoints of the standards, which assess the clinical susceptibility, was investigated. When the natural population was sensitive or intermediate according to the cited standard, it was described as naturally sensitive or naturally intermediate, respectively. When the natural population was clinically resistant, it was described as naturally (intrinsically) resistant. (The method in detail has been described previously [13–15].)

It should be noted that clinical breakpoints have not been defined by the most popular standards to include *Listeria* species. Apart from aminopenicillins and penicillin G, for which clinical breakpoints for *Listeria* susceptibility testing are defined according to the National Committee for Clinical Laboratory Standards (NCCLS) [16], in the present study the NCCLS criteria for staphylococci were applied [17]. For those antibiotics for which neither *Listeria* nor *Staphylococcus* assessment criteria were available, breakpoints for sensitivity were defined as follows: streptomycin, ≤ 8 mg/L; neomycin, ≤ 8 mg/L; spectinomycin, ≤ 32 mg/L; apramycin, ≤ 16 mg/L; ribostamycin, ≤ 16 mg/L; lividomycin A, ≤ 16 mg/L; piperacillin, ≤ 8 mg/L; ticarcillin, ≤ 4 mg/L; mezlocillin, ≤ 4 mg/L; azlocillin, ≤ 4 mg/L; cefotiam, ≤ 8 mg/L; cefixim, ≤ 8 mg/L; ceftibutene, ≤ 32 mg/L; biapenem, ≤ 4 mg/L; aztreonam, ≤ 8 mg/L; pefloxacin, ≤ 1 mg/L; pipemidic acid, ≤ 4 mg/L; roxithromycin ≤ 1 mg/L; lincomycin, ≤ 2 mg/L; fosfomycin, ≤ 64 mg/L; and fusidic acid, ≤ 2 mg/L. The MIC breakpoints for streptomycin, neomycin, spectinomycin, pefloxacin, pipemidic acid and lincomycin were chosen to be similar to French standards; the MIC breakpoints for roxithromycin, fosfomycin and fusidic acid correspond to Swedish assessment criteria. The majority of the remaining breakpoints (e.g. breakpoints for several β-lactams) are similar to the NCCLS criteria valid for Enterobacteriaceae.

RESULTS

Although there were no significant differences in natural susceptibility to numerous antibiotics among the species, there were others to which species-specific susceptibilities were detected. All *Listeria* species were naturally sensitive or intermediate to tetracyclines, aminoglycosides, penicillins (except for oxacillin), first- and second-generation cephalosporins (cefaclor, cefazolin, loracarbef), cefotiam, cefoperazone, carbapenems, macrolides, lincosamides, glycopeptides, dalfopristin/quinupristin and chloramphenicol. A natural sensitivity of all species was also shown to rifampicin, although the natural population of *L. grayi* was not clearly ascertainable. *Listeria* spp. were naturally resistant or intermediate to most 'modern' cephalosporins (cefetamet, cefixime, ceftibutene, ceftazidime, cefdinir, cefpodoxime, cefotaxime, ceftriaxone, cefuroxime; see below), aztreonam, pipemidic acid, dalfopristin quinupristin and sulfamethoxazole. Significant differences in natural susceptibility among the species were seen with the fluoroquinolones, trimethoprim, co-trimoxazole, rifampicin, fosfomycin and fusidic acid. *L. grayi* was naturally resistant to trimethoprim and co-trimoxazole, least susceptible to rifampicin and most susceptible to quinolones, whereas *L. ivanovii* was naturally resistant to most quinolones. *L. ivanovii* was naturally sensitive to fosfomycin, whereas *L. innocua* and *L. monocytogenes* were naturally resistant. *L. ivanovii* was also the most susceptible species to fusidic acid.

An overview of the antibiotic susceptibilities of the tested *Listeria* strains is presented in Figure 1. The MICs of antibiotics are presented separately for each species for which a distinctive pattern was demonstrated (Figure 1). The natural antibiotic sensitivities and primary resistances of *Listeria* spp. are summarized in Figure 2.

Medium dependency in susceptibility testing

For most of the tested antibiotics, there were no or minor differences in susceptibility dependent on the medium. These antibiotics included the aminoglycosides, most β-lactam antibiotics, quinolones, tetracyclines, streptogramins, chloramphenicol, antifolates, nitrofurantoin, fosfomycin, rifampicin and fusidic acid. For all the species, the MICs of macrolides were generally two dilution steps higher in CAMHB than in H-Medium; conversely, glycopeptide MICs were two to three dilution steps higher in H-Medium (data not shown). These differences did not affect the clinical assessment of natural susceptibilities. However, there were some cephalosporins for which there were medium-dependent differences in susceptibility partly affecting clinical assessment. Generally, for these antibiotics the chosen test strains showed higher susceptibilities in H-Medium, but the extent of these discrepancies differed among the species. The greatest medium-dependent differences were seen with some *L. welshimeri* strains, which were resistant to cefdinir (MICs 8–32 mg/L) and highly resistant to cefpodoxime, cefotaxime, ceftriaxone and cefuroxime in Mueller–Hinton medium (MICs > 64 mg/L), while being intermediate to cefdinir and having MICs of 4–16 mg/L to the latter cephalosporins in H-Medium (data not shown). Similar phenomena were seen with *L. seeligeri* and—to a lesser extent—with *L. grayi*. Minor medium-dependent susceptibilities to the mentioned cephalosporins were seen with *L. innocua*, *L. monocytogenes* and *L. ivanovii* strains. However, because of more clarity and the clinical significance of the latter species, all listeriae were described as being naturally resistant to cefdinir, cefpodoxime, cefotaxime, ceftriaxone and cefuroxime (see above and Figure 2).

Antibiotic (standard)	Concentrations examined (mg/L)	Taxon	Number of strains with MIC (mg/L) of																
			0.01	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Tetracyclines																			
Tetracycline (2)	0.03–64	All strains							13	31	42	12			1	4			
Doxycycline (2)	0.03–64	All strains				2	38	47	11					1	4				
Minocycline (2)	0.03–64	All strains				1	31	61	5					1	4				
Aminoglycosides																			
Amikacin (2)	0.13–256	All strains						5	27	24	29	15	2						
Gentamicin (2)	0.06–128	All strains			5	18	20	40	18	2									
Netilmicin (2)	0.06–128	All strains			5	18	24	42	12	1	1								
Tobramycin (2)	0.06–128	All strains				14	23	31	31	4									
Streptomycin (3)	0.13–256	All strains							2	5	31	26	34	3				2	
Kanamycin (2)	0.13–256	All strains						8	28	29	31	7							
Neomycin (3)	0.13–256	All strains				1	10	22	26	32	11		1						
Spectinomycin (3)	0.13–256	All strains										3	31	40	19	5	3	2	
Apramycin (3)	0.06–128	All strains						1	12	28	31	28	2	1					
Ribostamycin (3)	0.06–128	All strains							11	25	37	27	3						
Lividomycin A (3)	0.06–128	All strains						1	5	16	19	27	26	9					
β-Lactams: penicillins																			
Penicillin G (1)	0.01–32	All strains			14	41	42	6											
Oxacillin (2)	0.03–64	All strains					1	2	15	45	36	4							
Amoxycillin (1)	0.06–128	All strains			13	31	50	9											
Amoxycillin / Clavulanate (1)	0.06–128	All strains			14	37	44	8											
Ampicillin / Sulbactam (1)	0.06–128	All strains			8	25	39	29	2										
Piperacillin (3)	0.13–256	All strains					3	18	27	48	7								
Piperacillin / Tazobactam (2)	0.13–256	All strains			2	1	12	24	22	38	3								
Ticarcillin (3)	0.13–256	All strains					2	28	36	35	2								
Mezlocillin (3)	0.13–256	All strains				5	25	24	35	14									
Azlocillin (3)	0.13–256	All strains					46	37	20										
β-Lactams: cephalosporins																			
Cefaclor (2)	0.13–256	All strains							1	3	30	33	35	1					
Cefazoline (2)	0.13–256	All strains						10	37	46	7	3							
Loracarbef (2)	0.13–256	All strains							1	2	7	53	40						
Cefuroxime (2)[a]	0.03–64	*L. innocua*							1					1	8	11			
		L. monocytogenes										1	2	5	9	4			
		L. seeligeri									2	16	3						
		L. ivanovii									2	4	1		4	8			
		L. welshimeri							1		1	2	1	1	2	3			
		L. grayi								1	1	2	2	3	1				
Cefotiam (3)	0.03–64	All strains						1	16	62	18	3	2	1					
Cefetamet (3)	0.03–64	All strains												1	2	100			
Cefoxitin (2)	0.03–64	All strains								1	2	4	28	35	33				
Cefixim (3)	0.03–64	All strains										3	3	11	18	68			
Cefpodoxime(2)[a]	0.03–64	*L. innocua*							1					3	10	7			
		L. monocytogenes										3	6	9	3				
		L. seeligeri									4	17							
		L. ivanovii								1	5	2		1	2	8			
		L. welshimeri								1	2	2	2	1	2	1			
		L. grayi							1			4	1	4					
Cefdinir (2)	0.03–64	All strains					1	2	9	29	27	22	10	3					
Cefoperazone (2)	0.03–64	All strains							1	1	8	28	55	8	2				
Cefotaxime (2)[a]	0.03–64	All strains							2	1	4	22	21	20	18	15			
Ceftibutene (3)	0.03–64	All strains													1	102			
Ceftriaxone (2)[a]	0.03–64	All strains								2	1	8	25	15	28	24			

Figure 1 Antibiotic susceptibility of *Listeria* spp.
[a]MIC values differ significantly from those obtained in CAMHB (see results).

Antibiotic (standard)	Concentrations examined (mg/L)	Taxon	Number of strains with MIC (mg/L) of																
			0.01	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
β-Lactams: cephalosporins *(continued)*																			
Ceftazidime (2)	0.03–64	All strains												2	4	97			
Cefepime (2)	0.03–64	All strains									4	11	25	33	16	14			
β-Lactams: carbapenems																			
Imipenem (2)	0.03–64	All strains		2	7	26	35	25	8										
Meropenem (2)	0.03–64	All strains		4	39	45	15												
Biapenem (3)	0.03–64	All strains		23	42	36	2												
β-Lactams: monobactams																			
Aztreonam (3)	0.03–64	All strains											1	2	8	92			
Quinolones																			
Ciprofloxacin (2)	0.01–32	*L. innocua/ L. ivanovii/ L. seeligeri*							2	49	9	1							
		L. monocytogenes/ L. welshimeri						6	17	7	1	1							
		L. grayi				3	6		1										
Sparfloxacin (2)	0.01–32	*L ivanovii*									15	4							
		L. innocua/ L. seeligeri								19	23								
		L. monocytogenes/ L. welshimeri						2	15	14				1					
		L. grayi			2	5	2		1										
Norfloxacin (2)	0.03–64	*L. innocua/ L. ivanovii/ L. seeligeri*									2	42	10	7					
		L. monocytogenes/ L. welshimeri								1	18	8	3	1		1			
		L. grayi							7	2		1							
Ofloxacin (2)	0.01–32	*L. innocua/ L. ivanovii/ L. seeligeri*									53	8							
		L. monocytogenes/ L. welshimeri							5	21	5				1				
		L. grayi					2	7		1									
Enoxacin (2)	0.01–32	*L. innocua/ L. ivanovii/ L. seeligeri*										1	39	20	1				
		L. monocytogenes/ L. welshimeri									4	14	13	1					
		L. grayi							4	5		1							
Fleroxacin (2)	0.01–32	*L. ivanovii/ L. seeligeri*										33	7						
		L. innocua									14	7							
		L. monocytogenes/ L. welshimeri								9	19	3		1					
		L. grayi						2	7		1								
Pefloxacin (3)	0.01–32	*L. ivanovii/ L. seeligeri*										32	8						
		L. innocua								1	5	15							
		L. monocytogenes/ L. welshimeri								8	18	5		1					
		L. grayi						4	5		1								
Pipemidic acid (3)	0.06–128	*L. innocua/ L. ivanovii/ L. seeligeri*														1	58		
		L. monocytogenes/ L. welshimeri													2	7	24		
		L. grayi											2	7			1		
Macrolides																			
Erythromycin (2)[a]	0.03–64	All strains		1	8	61	32	1											
Roxithromycin (3)[a]	0.03–64	All strains			1	25	72	5											
Clarithromycin (2)[a]	0.03–64	All strains		14	64	24	1												
Azithromycin (2)[a]	0.03–64	All strains			1	11	59	28	4										
Lincosamides																			
Lincomycin (3)	0.01–32	All strains						2	18	29	23	20	2	1	8				
Clindamycin (2)	0.01–32	All strains					5	39	30	23	5	1							
Streptogramins																			
Dalfopristin (2)	0.03–64	*L. innocua/ L. grayi*														25			
		L. monocytogenes													7	14			
		L. welshimeri											1	1	5	4			
		L. ivanovii/ L. seeligeri										2	11	24	2				
Quinupristin (2)	0.03–64	All strains									9	31	41	21	1				
Dalfopristin/ Quinupristin (2)	0.03–64	All strains					2	3	53	35	9	1							

Figure 1 *continued*

Antibiotic (standard)	Concentrations examined (mg/L)	Taxon	Number of strains with MIC (mg/L) of																
			0.01	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Antifolates																			
Sulfamethoxazole (2)	0.25–512	All strains														1	3	11	88
Trimethoprim (2)	0.03–64	*L. grayi*													1	9			
		L. ivanovii					5	9		1	1		1			2			
		Other listeriae			11	22	21	19	1										
Co-trimoxazole (2)	0.13–256	*L. grayi*																10	
		L. ivanovii								4	10		2	1		1	1		
		Other listeriae						1	13	31	29								
Glycopeptides																			
Teicoplanin (2)[a]	0.06–128	All strains					1	8	71	21	2								
Vancomycin (2)[a]	0.03–64	All strains						2	15	68	18								
Other antibiotics																			
Chloramphenicol (2)	0.06–128	All strains							1	17	54	31							
Nitrofurantoin (2)	0.13–256	All strains											6	14	45	37	1		
Rifampicin (2)	0.01–32	*L. grayi*							4		2		3	1					
		L. innocua			3	6	9	2	1										
		L. monocytogenes			10	10	1												
		L. seeligeri		8	12	1													
		L. welshimeri		9	1		1												
		L. ivanovii		19															
Fosfomycin (3)	0.13–256	*L. innocua*/ *L. monocytogenes*															3	40	
		L. seeligeri/ *L. welshimeri*													5	6	8	13	
		L. grayi												1	4	2	1	2	
		L. ivanovii								1	1	4	5	6	2				
Fusidic acid (3)	0.01–32	*L. seeligeri*										19	2						
		L. ivanovii						2	2	8	3		4						
		Other listeriae				1						9	43	10	1				

[a]MIC values differ significantly from those obtained in CAMHB (see results).
The number of strains for the corresponding MIC value is cited. A number in the lowest concentration of the antibiotic represents the maximal MIC value of this concentration ($MIC=C_{min} \rightarrow MIC \leq C_{min}$). A MIC value higher than the highest concentration tested is cited in the subsequent higher concentration step. MIC values in shaded areas indicate the clinically intermediate area according to the American standard (NCCLS) valid for *Listeria* spp. (indicated as (1) in the table) [16] or *Staphylococcus aureus* strains (indicated as (2)) [17]. A thick black line indicates the NCCLS breakpoint between the clinically sensitive and resistant strains, if the interpretation 'intermediate' does not exist. For antibiotics to which neither *Listeria* nor *Staphylococcus* NCCLS clinical assessment criteria exist, breakpoints for sensitivity were defined (indicated as (3); see Materials and Methods).
Figure 1 *continued*

DISCUSSION

The results of the present study show that all *Listeria* species currently known show similar natural susceptibility patterns to numerous antibiotics, but there are also several agents to which there are species-dependent differences in natural antibiotic susceptibility (Figure 2). This is in contrast to the widely held opinion based upon the studies of Espaze et al [18] and MacGowan et al [19], showing only species-dependent differences in susceptibility to fosfomycin.

Apart from *L. grayi*, listeriae possess typical natural susceptibility patterns to various antimicrobial agents expected for many Gram-positive bacteria, such as natural sensitivity to glycopeptides, tetracyclines, trimethoprim, penicillins, carbapenems, rifampicin, macrolides, lincosamides and chloramphenicol, decreased susceptibility to fluoroquinolones and natural resistance to most cephalosporins and non-fluoroquinolones. These data are in agreement with the data from the literature [1,19–23]; however, in most studies only a few strains of species other than *L. monocytogenes* or a small number of antibiotics had been tested, and there is little information on natural antibiotic susceptibility patterns.

Susceptibility patterns characteristic for the genus *Listeria* were reflected by the uniform natural aminoglycoside sensitivity of all species and the obvious natural resistance to sulfamethoxazole and 'modern' cephalosporins. In the literature, the activity of aminoglycosides against listeriae is well documented [24]. However, it is not known why in the present

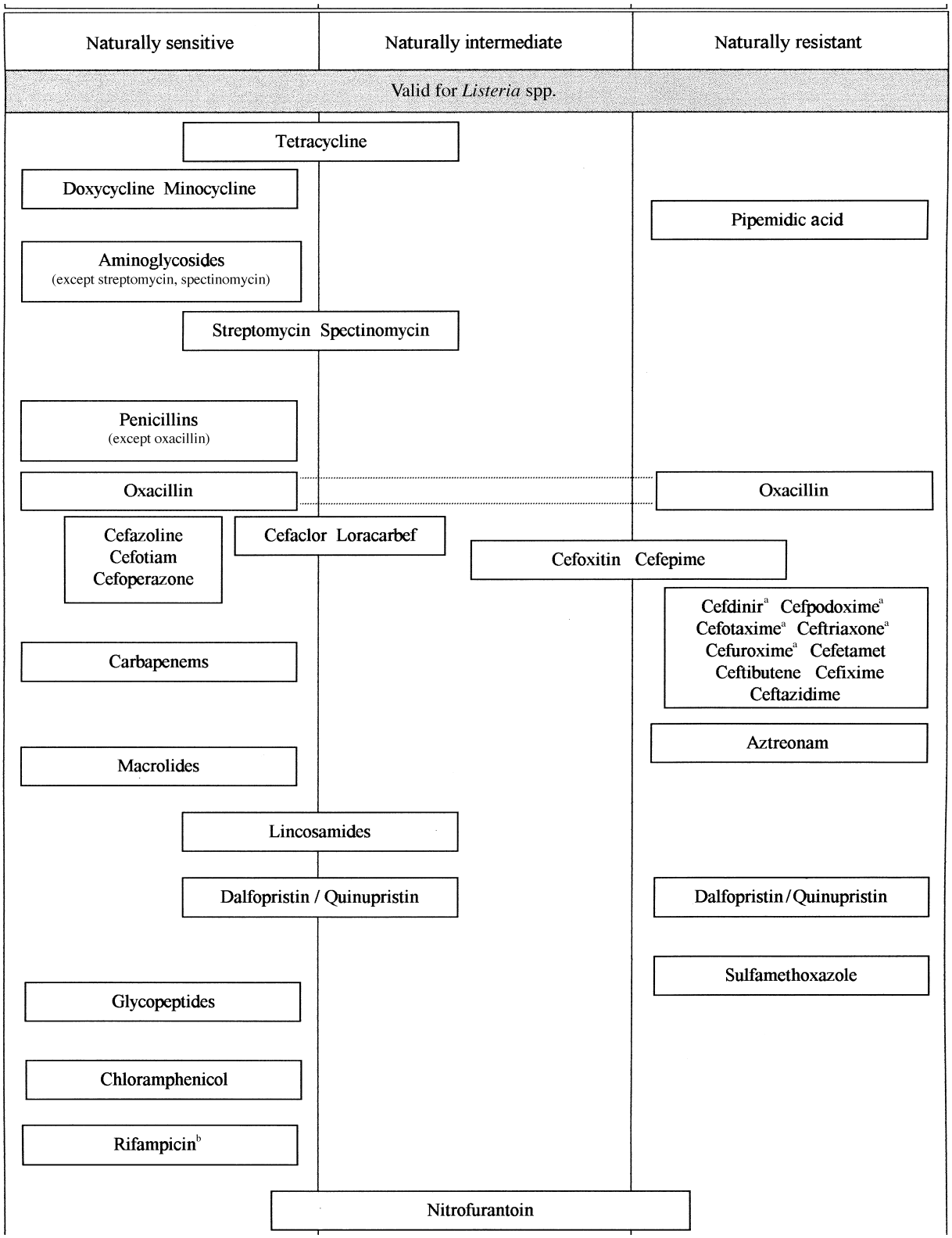

Figure 2 Natural antibiotic susceptibility of *Listeria* spp., classified as sensitive, intermediate and resistant according to the standards mentioned in Figure 1.
[a]See Results.[b]The natural populations of *Listeria grayi* susceptible to rifampicin and fosfomycin were not unequivocally ascertainable.[c]See Discussion. Note: If less than 10% of the strains belonging to a natural population were attributable to one of the clinical categories, these strains were not taken into consideration.

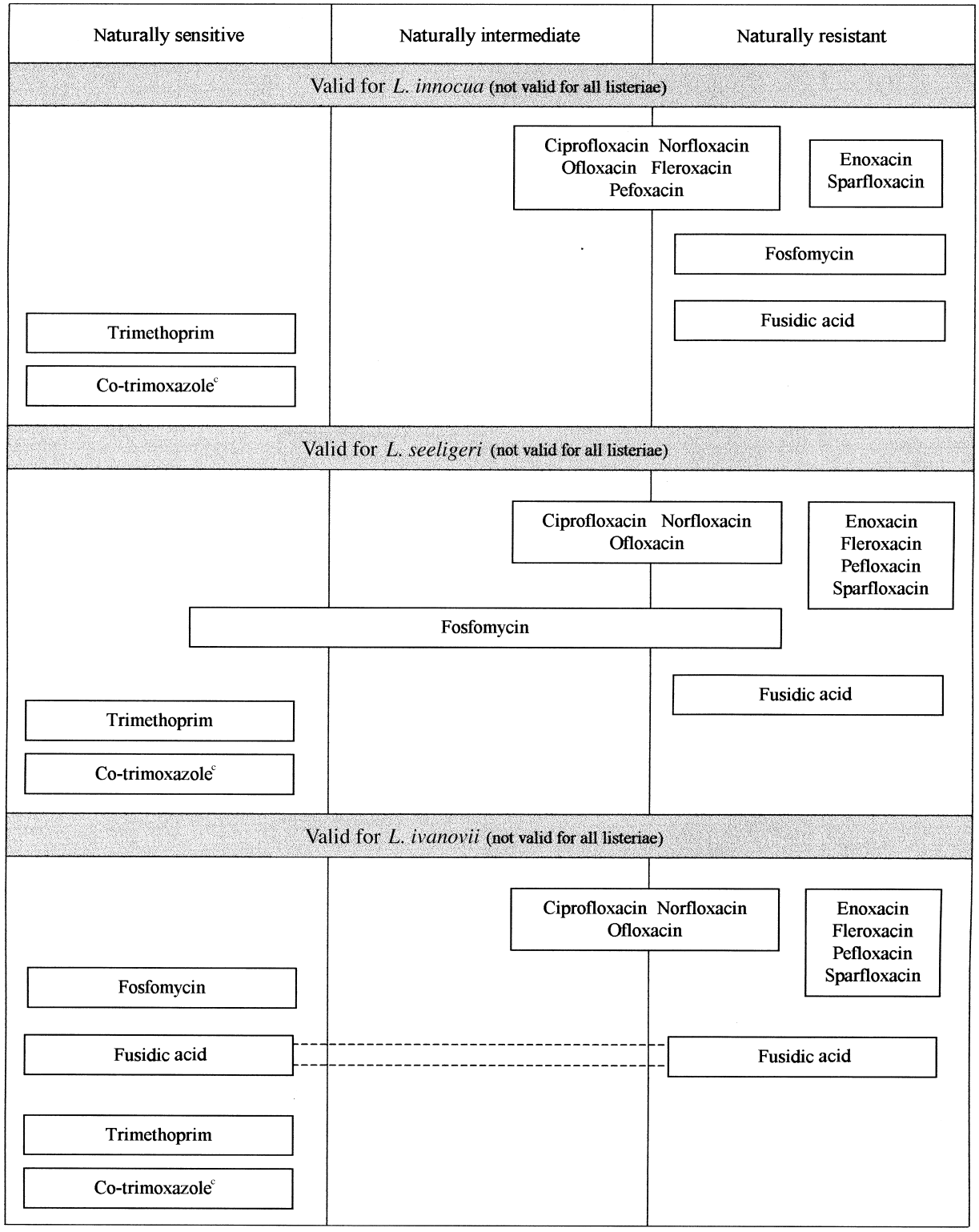

Figure 2 *continued*

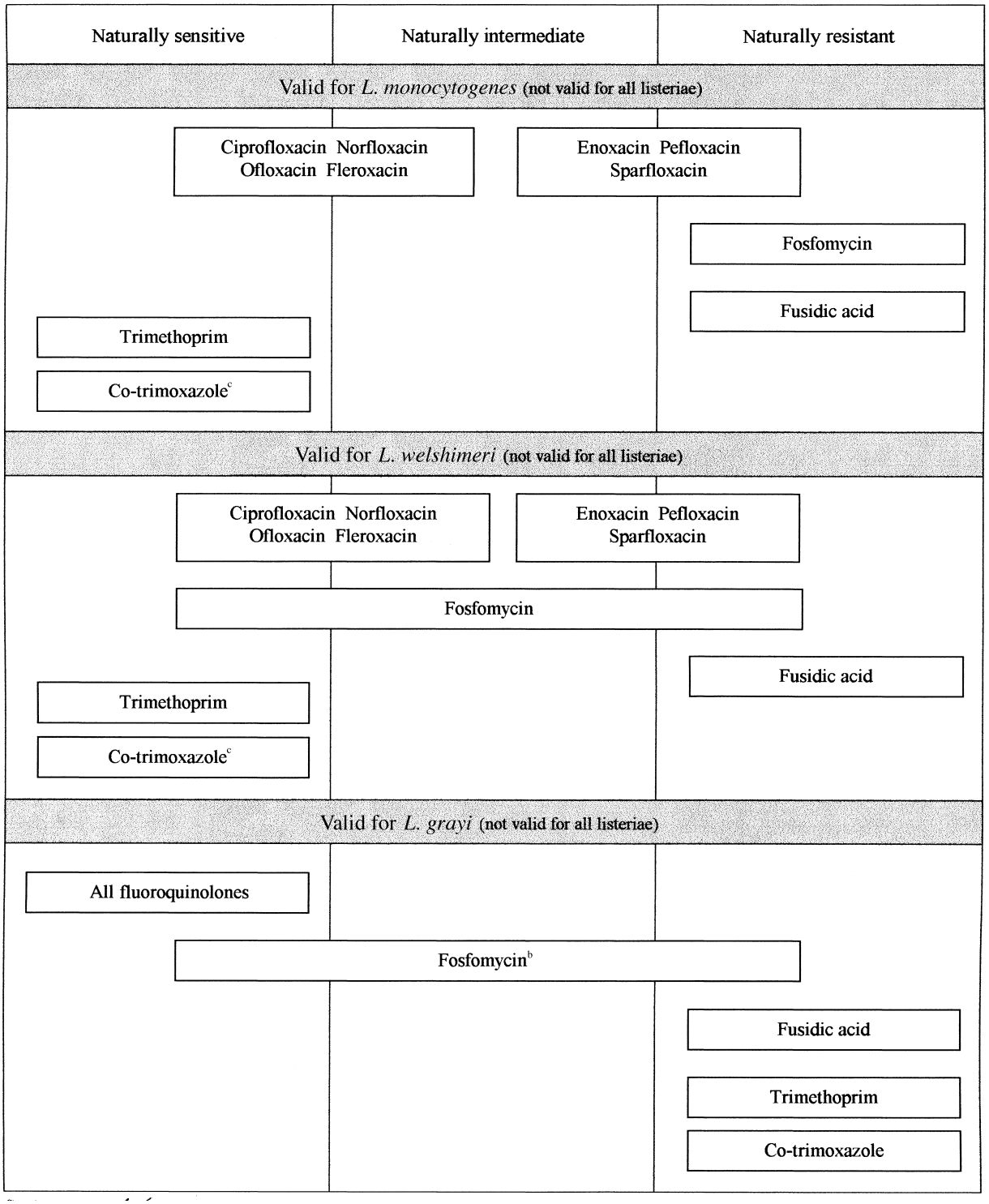

Figure 2 *continued*

study all listeriae were highly resistant to sulfamethoxazole in both H-Medium and CAMHB, whereas Hof et al found some activity of sulfamethoxazole against 13 *L. monocytogenes* strains using Mueller–Hinton medium for susceptibility testing [24]. Although it cannot be excluded that probable differences in the cation and anion composition of the media have contributed to the observed phenotypes, the background of the observed discrepancies remains obscure.

Natural resistance to broad-spectrum cephalosporins associated with natural sensitivity to older cephalosporins is a phenomenon also found in lactobacilli and *Bacillus* spp. and is associated with the lack of appropriate penicillin-binding proteins (PBPs) in the *Listeria* cytoplasmic membrane [24]. *Listeria* spp. possess five PBPs that are characterized by different molecular masses [24]. PBP3 is considered to be the primary target for active β-lactams in *Listeria*, since enzyme blockage has lethal consequences for the bacterial cell [25]. Broad-spectrum cephalosporins, as well as monobactams, do not bind with high affinity to PBP3, whereas several penicillins (and probably some first- and second-generation-cephalosporins) do [24].

An interesting aspect of the present study is the *Listeria* susceptibility pattern to co-trimoxazole. First-choice treatment of listeriosis generally consists of ampicillin combined with gentamicin, or of co-trimoxazole [26,27]. Whereas good activity for the former antibiotics was confirmed, our data suggest that approximately half of all listeriae except for *L. grayi* were naturally low-level resistant to co-trimoxazole (Figure 1). This finding is surprising, especially because co-trimoxazole—apart from its use in combination with an aminopenicillin—is the second choice for the treatment of listeria infections [27], and resistance to antifolates (except sulfamethoxazole) in 'pathogenic' listeriae is very rare [20]. Thus, our results primarily point to the lack of suitable NCCLS breakpoints for co-trimoxazole and *Listeria* spp. The NCCLS breakpoint defined for staphylococci (see Figure 1 [17]) separates the natural population of all *Listeria* species except that of *L. grayi*, leading to 39 additional 'resistant' strains. Changing the NCCLS breakpoint for resistance from ≥ 4 mg/L to ≥ 8 mg/L would correspond to 100% co-trimoxazole sensitivity for all *Listeria* strains not belonging to *L. ivanovii* or *L. grayi* (Figure 1). Another point taken into consideration is the thymidine content of the applied media. H-Medium and CAMHB both contain thymidine, which antagonizes the activity of co-trimoxazole and trimethoprim [28]. However, because all the appropriate strains were highly susceptible to trimethoprim (Figure 1), the thymidine content of the applied media is likely to be of secondary significance for the observed phenotype.

A new insight into natural antibiotic resistance is the obvious resistance of *L. grayi* strains to all antifolates. Although only 10 strains were tested, it seems likely that the observed antifolate patterns are a natural phenomenon, favored by the uniformity in susceptibility and the selected strains originating from different countries and different sources (see Materials and methods). Secondary resistance to trimethoprim in *L. monocytogenes* was recently attributed to a 3.7-kb plasmid (pIP823) containing a gene (*dfrD*) coding for a high-level trimethoprim-resistant dihydrofolate reductase [29]. Because the host range of pIP823 was shown to be broad, and conjugative mobilization of pIP823 was obtained by self-transferable plasmids between different species [30], it is likely that a stable pIP823 or a chromosomally encoded sequence containing or resembling *dfrD* occurs in *L. grayi* strains. The unique natural susceptibility patterns of *L. grayi* among *Listeria* spp. to trimethoprim and co-trimoxazole, quinolones and rifampicin confirm its status as a *Listeria* species showing a separate line of descent [3,4] and support the assignment of '*L. murrayi*'—whose type strain was included in the present study—as a subspecies of *L. grayi*.

In the coming years, several studies will be needed to elucidate the mechanisms contributing to the observed species-specific natural antimicrobial susceptibilities among *Listeria* species. It might be possible that variations in the compositions of the cell walls contribute to the differences in susceptibility to the fluroquinolones, fusidic acid, fosfomycin and rifampicin. It can be assumed that there are particular uptake mechanisms for fosfomycin and fusidic acid in *L. ivanovii* which are absent in other listeriae. The clinical significance of these findings has yet to be proven.

In conclusion, the present study represents a database for the natural susceptibility of *Listeria* spp. to a wide range of antibiotics. This database can be used for the validation of antibiotic susceptibility test results of *Listeria* spp., especially with those antibiotics to which the species are naturally resistant. If *Listeria* strains are found that are sensitive to these antibiotics, antibiotic susceptibility testing should be repeated and identification results confirmed. It has to be elucidated whether the present study might extend the possibilities for the effective treatment of *Listeria* infections.

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