Large Nationwide Outbreak of Invasive Listeriosis Associated with Blood Sausage, Germany, 2018–2019

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RESEARCH

Exceptionally large and country-wide outbreak of invasive listeriosis associated with blood sausage consumption, Germany 2018-2019

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48 **ABSTRACT**

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50 Invasive listeriosis is a severe food-born infection in humans with increasing incidence world-
51 wide. The disease is difficult to control from the public health perspective, but molecular
52 surveillance programs have been implemented by different countries for improving recognition
53 and management of listeriosis outbreaks. Routine whole genome sequencing (WGS), core
54 genome multi locus sequence typing (cgMLST) and single nucleotide polymorphism (SNP)
55 calling were used for subtyping of *L. monocytogenes* isolates from listeriosis cases and suspected
56 foods in Germany. This identified an unusually large cluster of *L. monocytogenes* isolates with
57 134 highly clonal, benzalkonium-resistant ST6 isolates collected in 2018-2019 and allocated to
58 112 notified listeriosis cases. Epidemiological investigations identified blood sausage as the
59 outbreak vehicle, which was contaminated with isolates highly related to the clinical isolates.
60 Withdrawal of the product from the market ended the outbreak. This cluster represents one of the
61 largest European outbreaks of invasive listeriosis in the last 25 years.

INTRODUCTION

Listeriosis is a severe, mainly foodborne human infection associated with high case fatalities and hospitalization rates when compared to other bacterial gastrointestinal pathogens (1). The causative agent, *Listeria monocytogenes*, occurs ubiquitously in the environment and disseminates into the food production chain. Patients develop either self-limiting non-invasive gastroenteritis (2, 3) or invasive listeriosis. Listeriosis affects mainly elderly, immunocompromised patients and pregnant women, causing a severe invasive form of the disease leading to sepsis, meningitis, and encephalitis or neonatal infections, and miscarriage (4). Case fatality rates of invasive listeriosis are around 30% for neurolisteriosis and even higher in septic patients (5). In Europe and North America, invasive listeriosis affects 0.3-0.6 patients per 100,000 inhabitants per year (6, 7).

L. monocytogenes forms hard-to-remove biofilms in food-processing plants, can acquire tolerance to sanitizers and multiplies even at refrigeration temperatures (8). These properties complicate efficient prevention of *L. monocytogenes* contaminations in different types of ready-to-eat products: dairy, meat and fish as well as in fruits and vegetables, all of which have caused listeriosis outbreaks in the past (9-12).

Outbreaks of listeriosis are difficult to control for several reasons: First, case numbers are low, impairing the generation of valid hypotheses on possible food sources through patient interviews. Second, incubation time can be lengthy (1-67 days) (13), and patients are often seriously ill, further complicating patient interviews. Third, the large variety of possible food sources makes pinpointing through patient interviews and the follow-up tracing of food difficult. Moreover, listeriosis outbreaks can be geographically widespread due to long-distance food trade connections, e-commerce, and traveling, thus hampering outbreak recognition by local authorities

(10, 14, 15). Finally, listeriosis outbreaks can be protracted and may last for several years (16), making it difficult to identify affected patient groups correctly and to allocate the common source of infection.

Nationwide systematic collection of *L. monocytogenes* isolates from human listeriosis cases and their subtyping using high-resolution WGS-based typing techniques allows for rapid and reliable detection of outbreak clusters (3, 17-22), some of which were not detectable in the past. At the same time, systematic and on-demand typing of food-associated *L. monocytogenes* isolates assists in detecting outbreak sources. In a recent French molecular surveillance study, one-third of all isolates were grouped into WGS clusters and the majority of these clusters contained less than 5 isolates (20). Larger outbreaks of invasive listeriosis occur, although infrequently, and two of the world´s largest outbreaks in the recent past included 147 cases in a multi-state US outbreak (2011) associated with cantaloupe melons (10) and 1,060 cases in a South African outbreak (2017-2018) associated with French polony sausage (11). Since August 2019, Spain has been experiencing another large listeriosis outbreak (23). However, the scientific evaluation of this outbreak needs to be awaited.

Here we present an exceptionally large nation-wide outbreak with 134 laboratory-confirmed isolates allocated to 112 patients with epidemiological investigations and complementary WGS-based typing of food isolates identifying the outbreak vehicle. This outbreak represents one of the largest outbreaks of invasive listeriosis in Europe documented in the scientific literature during the last 25 years.

METHODS AND MATERIALS

Bacterial strains and growth conditions.

All 184 *L. monocytogenes* isolates are listed in Table S1. *L. monocytogenes* strains were routinely cultured at 37°C in brain heart infusion (BHI) broth, on BHI or sheep blood agar plates.

Isolation of food isolates

Detection and enumeration of *L. monocytogenes* from food samples was performed according to EN ISO 11290 parts 1 and 2. The species was confirmed according to ISO standard 11290-1:2017 or MALDI-TOF-MS, and a previously described multiplex PCR (24).

Determination of molecular serogroups

The GenElute Bacterial Genomic DNA Kit (Sigma) or the QIAamp DNA Mini Kit (Qiagen) was used to isolate chromosomal DNA. Molecular serogroups were determined by multiplex PCR (25).

Genome sequencing, multi locus sequence typing (MLST) and core genome MLST (cgMLST)

DNA was quantified using the Qubit dsDNA BR (or HS) Assay kit and Qubit fluorometers (Invitrogen). Libraries were prepared using the Nextera XT DNA Library Prep Kit (Illumina). Isolates were sequenced on MiSeq, HiSeq, or NextSeq sequencers. Raw reads were trimmed and assembled in SeqSphere using the Velvet assembler. *In silico* serogroups, MLST sequence types (STs) and 1,701 locus cgMLST complex types (CTs) were extracted using the Ridom SeqSphere Software through automated allele submission to the *L. monocytogenes* cgMLST server

(http://www.cgmlst.org/ncs/schema/690488/) (26). Coverage ranged between 22- and 116-fold (median 54-fold). cgMLST clusters were defined as groups of isolates with ≤10 different alleles between neighboring isolates. UGPMA trees were calculated in SeqSphere in the "pairwise ignore missing values" mode.

SNP-based alignments

Mapping of sequencing reads, generation of consensus sequences, alignment calculation, and SNP filtering (exclusion distance = 300) were performed using in-house pipelines (17). The 10-092876-0769 LM12 genome (NZ_CP019625, serogroup IVb, ST6, CT6304) was used as reference. Generation of maximum likelihood trees was performed using the Geneious 9.1.3 Tree builder (Biomatters Ltd.) and the RAxML plugin.

Virulome and resistome analysis

Virulence and resistance genes of *L. monocytogenes* were included as target loci in SeqSphere task templates as previously described (17, 27). Targets were extracted from assembled contigs using SeqSphere, and alleles were considered to be present when identity was >90% and at least 99% of the reference sequence aligned with the query sequence.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed by a microdilution assay in a 96 well plate format in accordance with EUCAST guidelines in Mueller Hinton Fastidious (MH-F) broth (28). The overall plate design was adopted from a study by Noll et al. (29).

153 **Determination of tolerance to disinfectants**

154 *L. monocytogenes* isolates were spread on BHI agar plates. Cellulose discs (6 mm in diameter)
155 were loaded with 10 µl of a 10 mg/ml aqueous benzalkonium chloride solution and placed on top
156 of the agar plate. Plates were incubated overnight at 37°C and growth inhibition zone diameters
157 were determined. Statistical significance was tested by Student's *t*-test.

158

159 **Case-control study**

160 Outbreak cases were defined as patients reported to public health authorities and transmitted to
161 the Robert Koch Institute with disease onset between August 2018 and June 2019, with isolation
162 of *L. monocytogenes* from normally sterile body fluids or in birth settings and confirmation by
163 cgMLST and SNP-analysis. *L. monocytogenes* isolates were sent to the Robert Koch Institute and
164 notification and typing data were merged for investigation. After identification of the outbreak,
165 patients were interviewed using a standardized questionnaire on food consumption during the two
166 weeks before the onset of illness, general eating habits, and food purchasing behaviors. Based on
167 these data, 40 food items were included in the case-control study. Controls were contacted and
168 interviewed by a survey institute. The controls were frequency matched to the case patients for
169 age, gender and place of residence (federal state). Food items with p-value ≤ 0.05 that were
170 consumed by more than 50% of the subjects were considered for multivariable analysis. The
171 stepwise-backward approach for model formation was that food items, which were no longer
172 significantly associated, were consecutively excluded from the multivariable model until only
173 significantly associated foods and their confounders remained. Risk measures (odds ratios,
174 univariable and multivariable) were determined in the statistical analysis.

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Data availability

Genome sequences are available at the European nucleotide archive (https://www.ebi.ac.uk/ena). Accession numbers are listed in Table S1.

RESULTS

Detection of a large human listeriosis cluster by molecular surveillance

The binational German-Austrian Consultant Laboratory for *L. monocytogenes* collects isolates from approximately two-thirds of all mandatorily-notified German listeriosis cases (701 cases in 2018), and sequences their genomes. cgMLST identified a phylogenetically diverse cluster (designated "Epsilon1") with 46 PCR serogroup IVb isolates belonging to MLST ST6 and cgMLST complex types (CT) 90, 2981, 3803, 3805, 3806, 3921, 4083, 4465, 6236, 6331, 7353, and 7451, all defining specific allelic profiles within a CT threshold of ≤10 different alleles (17, 26). This cluster included isolates from 2011-2019 collected from all over Germany without any apparent geographical concentration. Allelic distances between these isolates varied from 0-25 (median: 11). In autumn 2018, a sudden increase of CT4465 and CT7353 isolates belonging to the Epsilon1 cluster was detected. Furthermore, the numbers of listeriosis cases reported in calendar weeks 34-43, 46, 48, and 50 exceeded the median of the five previous years (Fig. S1). To identify the outbreak clone among all incoming PCR serogroup IVb isolates, a clone-specific PCR was developed (supplementary information). Altogether, 134 clinical CT4465 and CT7353 isolates were collected between August 2018 and April 2019. These isolates formed a remarkably homogenous cluster with only 0-5 (median: 0) different cgMLST alleles (Fig. 1). In contrast, two earlier CT4465 isolates collected in June 2018 and July 2017 differed in 9-15 alleles (Fig. 1). Raw sequencing reads of all Epsilon1 strains were mapped against the 10-092876-0769 LM12 genome as the most closely related, completed genome available. SNP calling separated the Epsilon1 cluster into several sub-clusters, but all CT4465 and CT7353 isolates collected from August 2018 onwards formed a single cluster (Fig. S2). This sub-cluster was named Epsilon1a, and SNP distances within this cluster ranged between 0-3 SNPs (median: 0). The two earlier

CT4465 isolates were separated from the Epsilon1a cluster with 6-10 SNPs difference (median: 8). Thus, SNP calling supports the detection of a cluster of closely related CT4465 and CT7353 strains. Interestingly, only 21-29 cgMLST alleles (median 26) and 8-12 SNPs (median 8) differed between the Epsilon1a clone and the South African outbreak strain (CT5886, Fig. 1, Fig. S2).

Description of the patient collective

The 134 isolates could be allocated to 112 patients according to the case definition. Initial cases were reported in August 2018, with the outbreak peaking in September 2018 (Fig. 2A). The last case was notified in April 2019 (as of 25th March 2020). Cases occurred in 11 of 16 federal states in Germany with the majority of the cases occurring in Western and Southern Germany (Fig. 2B). This outbreak and the assembled genome of one representative isolate (isolate ID: 18-04540) were communicated via the European Epidemic Intelligence Information System (EPIS) platform on 23rd October 2018 (UI-516). France, as the only other country involved, reported an Epsilon1a listeriosis patient who had travelled to Germany and purchased food there. Sequence data of isolate 18-04540 was submitted to ENA (SAMEA5041142). The closest related isolate available at the National Center for Biotechnology Information (NCBI) pathogen detection pipeline was a Dutch clinical isolate from 2016 with a SNP distance of 12, which was clearly above the SNP distances observed within the Epsilon1a cluster.

There were 66 men (59%) among 111 non-pregnancy-associated outbreak patients. Seven patients died (6.3%), two of them with listeriosis as the primary cause. One Epsilon1a isolate originated from a pregnant woman (0.9%). Gestational age and health outcome of the newborn were not reported. The remaining isolates came from adult patients aged 53-98 years (median 79 years). The age distribution was not noticeably different from other notified listeriosis cases. Of the 134 Epsilon1a isolates, 99 were isolated from blood samples, 13 from cerebrospinal fluid, one

each from lymph nodes, ascites, sputum, pleura, joints, abscesses, or a superficial wound (Table S1). The type of isolation source for the remaining 15 isolates is not known.

Properties of the outbreak clone

Virulome analysis revealed the presence of *Listeria* pathogenicity island 1 (LIPI-1) in all Epsilon1a outbreak isolates and detected the complete listeriolysin S-encoding pathogenicity island LIPI-3 in 64% of them. In contrast, LIPI-4, encoding a putative phosphotransferase system associated with neurolisteriosis (30), was not present (Fig. S3). The Epsilon1a clones carried the same complement of internalin genes as other PCR serogroup IVb strains (Fig. S3). Susceptibility testing revealed sensitivity towards most clinically relevant antibiotics, however all tested isolates were fully resistant to ceftriaxone and daptomycin (Tab. S2). This is consistent with the intrinsic resistance of *L. monocytogenes* and the absence of additional resistance determinants, as suggested by the resistome approach (Fig. 3A). Further resistome analysis demonstrated the prevalence of the *emrC* gene associated with benzalkonium chloride tolerance (Fig. 3A). In full agreement with this observation, increased tolerance of the Epsilon1a and Epsilon1 isolates to benzalkonium chloride was shown when compared to other ST6 or PCR serogroup IVb isolates not belonging to the Epsilon1/1a clusters (Fig. 3B).

Investigations for identifying the outbreak vehicle

In our case-control study, 41 cases and 155 controls were included. A total of 40 out of 41 (98%) cases reported that they had purchased food in stores of one specific supermarket chain, in contrast to 99 out of 154 controls (odds ratio 22.5; p-value = 0.003, 95% CI: 2.9-174.9). Other supermarket chains were not associated with these cases. Thus, only cases and controls that had purchased food from this specific supermarket were included in further analyses. In the fourth

252 calendar week of 2019, a strong association of cases with the consumption of minced meat (odds
253 ratio of 42.4; p-value = 0.001, 95% CI: 4.3-415.4) and blood sausage (odds ratio of 23.1; p-value
254 = <0.001, 95% CI: 4.3-123.5; Table 1) was detected. A total of 90% of the cases reported having
255 consumed minced meat (45% of controls) and 80% of the cases blood sausage (23% of controls).
256 There were no vegetarians among the cases.
257 In order to perform risk-oriented screening, food samples were collected in supermarkets and the
258 households of some patients, according to the results of the epidemiological investigations. In one
259 case, *L. monocytogenes* could be detected in three open samples from a patient's refrigerator.
260 Among these, sliced blood sausage purchased at the incriminated supermarket chain showed the
261 highest contamination (>$3x10^6$ CFU/g). This led to another round of intensified screening of
262 prepacked blood sausage. In calendar week 7 (2019), *L. monocytogenes* was found in an original
263 sealed package of sliced blood sausage (<10 CFU/g) and in a second blood sausage sample of the
264 same manufacturer. In total, five isolates from patients' households food items and from blood
265 sausage samples grouped with clinical Epsilon1a isolates after cgMLST (0-3 different alleles,
266 median=0) and SNP calling (0-2 SNPs, median=0) (Fig. 1, Fig. S2). The blood sausage was
267 produced by a big German meat and sausage manufacturer and sold in large parts of the country.
268 The product was withdrawn from the market on 12th February 2019, and the last clinical
269 Epsilon1a isolate was collected on 18th April 2019. In contrast, Epsilon1 isolates not belonging to
270 the Epsilon1a cluster caused disease even after the end of the Epsilon1a outbreak. The plant was
271 cleaned and disinfected. Thereafter, *L. monocytogenes* was not detected in several hundred
272 official and ~2.500 own control samples taken from products or the production site.

DISCUSSION

The Epsilon1a outbreak is the largest outbreak of listeriosis ever identified in Germany and represents one of the largest outbreaks of invasive listeriosis in Europe documented in more than 25 years. The last reported European outbreak of invasive listeriosis of the same order of magnitude dates back to 1992-1993 when 247 patients were infected in France with a serotype 4b clone that contaminated pork tongue in aspic (31). Cantaloupe melons were the vehicle in the large US outbreak in 2011, which was caused by five different clones (10). In contrast, the single clone causing the world's largest outbreak in South Africa showed strong clonality. The genomes of 326 isolates from this outbreak only differed in ≤4 cgMLST alleles (11). Likewise, only a single clone was associated with the Epsilon1a outbreak, and high clonality was observed among its isolates. The mutation rate in the natural *L. monocytogenes* population is 2.6×10^{-7} substitutions/site/year (27) so that one SNP can be expected per year on average and genome for *L. monocytogenes* strains under natural conditions. The high clonality could imply that the outbreak clone may only have persisted in the production facility without rapid multiplication. Purchases in a particular supermarket chain and consumption of blood sausage were strongly associated with listeriosis in the case-control study and the outbreak clone was identified in blood sausage samples from a patient´s household and from the incriminated supermarket chain. Blood sausage is heat-treated during production, so contamination likely occurred post-production, possibly during slicing or packaging. The shelf-life of sliced blood sausage is short (several days to few weeks) (32), and the amount of *L. monocytogenes* found in unopened blood sausage samples was below the limit of 100 CFU/g. Storage beyond the anticipated shelf-life or insufficient refrigeration might have allowed *L. monocytogenes* multiplication inside the vehicle; this only could have been prevented by a zero tolerance policy. The type of the vehicle could also

297 explain the low number of pregnant women (1 out of 112) since typically pregnancy-related
298 listeriosis accounts for ~7% among all listeriosis cases (33): Pregnant women should be cautious
299 with sliced sausage according to official recommendations (34).
300 Analysis of the Epsilon1a genome has yielded some insights into the infectivity of this clone, as it
301 belongs to sequence type ST6. *L. monocytogenes* ST6 clones were first isolated in 1990 (35),
302 caused various outbreaks in the past, including the large South African outbreak, and are
303 associated with an unfavorable outcome of meningitis (36). The Epsilon1a clone and the South-
304 African outbreak strain are closely related. Thus, two descendants of the same historic *L.*
305 *monocytogenes* ancestor have spread globally and contaminated food production facilities on two
306 different continents. The Epsilon1a clone carried the *emrC* gene, which is the presumable cause
307 of its increased benzalkonium chloride tolerance (37). Benzalkonium chloride was banned in the
308 EU as a disinfectant in 2016 (38), but its use in the past might have selected tolerant strains.
309 The identification of this outbreak and its vehicle resulted from an efficient collaboration between
310 public health and food safety authorities in Germany. Several requirements had to be met first for
311 successful outbreak clarification: (i) developing a mandatory notification system to facilitate
312 systematic patient interviews with an efficient questionnaire to generate hypotheses on possible
313 food sources, (ii) implementing a WGS-based molecular surveillance program for reliable
314 identification of outbreak clusters by public health authorities, (iii) systematically collecting of
315 food isolates from internal controls and on-demand investigations and their subtyping using
316 harmonized WGS-based methodology by food safety authorities, and (iv) continuously
317 exchanging information on outbreak clusters between the institutions involved. With these
318 prerequisites at hand, the causative food vehicles for five out of the six biggest listeriosis clusters
319 that occurred in 2014-2019 in Germany have been identified, and it is questionable whether this
320 would have been possible in pre-WGS times. Nevertheless, routinely conducted interviews of

listeriosis cases regardless of outbreaks would probably have enabled a faster identification of the outbreak vehicle. In our opinion the course of the Epsilon1a outbreak is a good example to demonstrate how WGS-based pathogen surveillance combined with efficient interventions of the involved stakeholders can improve management and prevention of foodborne infectious diseases in general.

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FIGURE LEGENDS

Figure 1: Identification of the Epsilon1a outbreak cluster by cgMLST.

UPGMA tree calculated using cgMLST data of Epsilon1 isolates (red) and the Epsilon1a sub-cluster (green - clinical isolates, blue - food isolates). Two earlier CT4465 isolates (July 2017 and June 2018) not belonging to Epsilon1a are shown in pink. Strain 10-092876-0769 LM12 (used as reference genome for SNP calling) shown in Fig. S2 is included for comparison (black). South African outbreak isolates (11) are shown in orange.

Figure 2: Spatial and temporal distribution of outbreak cases.

(A) Diagram showing the number of Epsilon1a isolates received by the consultant laboratory per week. CW - calendar week. (B) Geographical distribution of laboratory-confirmed Epsilon1a cases within Germany.

Figure 3: Tolerance of Epsilon1a isolates to benzalkonium chloride.

(A) Resistome analysis of clinical *L. monocytogenes* Epsilon1 and Epsilon1a isolates. Assembled genome sequences were searched for genes known to confer resistance to sanitizers, heavy metals, and antibiotics using a SeqSphere task template. Genomes of strains EGD-e (NC_003210.1), L2624 (NZ_CP007686) FORC_049 (NZ_CP016629), 6179 (NZ_HG813249), LM201 (AYPT00000000), 2012-0070 (MNCF00000000), NCTC 10887 (MWLR00000000), 10-092876-0731 LM5 (NZ_CP019618), and 12754_4#74 (ERR564017) + pLMST6 (Hx2000053471) were included for comparison. Abbreviations: QACs - quaternary ammonium compounds, sm – streptomycin, cap – chloramphenicol, erm – erythromycin, fos – fosfomycin, lin – lincomycin, flu – fluoroquinolones, tet – tetracycline, cfx – ceftriaxone. Please note that the

emrC gene is located on plasmid pLMST6, which is present in certain ST6 strains (39). Its location on a plasmid may explain why it was not detected throughout the entire Epsilon1a population due to plasmid loss. (B) Increased resistance of Epsilon1a and Epsilon1 isolates to benzalkonium chloride. Three representative isolates belonging to human listeriosis clusters Epsilon1a, Epsilon1, and distinct listeriosis clusters Lambda2 (ST2, CT2402), Pi3 (ST217, CT5744) or Theta3 (ST249, CT4449) were tested for resistance to benzalkonium chloride by disc diffusion. Three representative ST6 isolates, not belonging to Epsilon1, were also included. Results of three independent replicates for all three isolates per group are shown. The asterisk indicates statistically significant differences to Epsilon1a ($P \leq 0.01$, *t*-test).

Table 1: Results of multivariable analysis of food consumption from a case-control study during the Epsilon1a listeriosis outbreak, Germany 2018-2019. Only 40/41 cases and 99/155 controls that confirmed shopping at the incriminated supermarket chain were included.

Food item	Odds Ratios (multivariable*)	95% Confidence intervall	p-value
Minced meat	42.4	4.3-415.1	0.001
Blood sausage	23.1	4.3-123.5	<0.001
Cold cuts (roast pork / Kassler)	15.4	2.9-82.1	0.001
Edamer cheese	7.3	1.6-32.8	0.009
smoked ham [#]	0.06	0.0-0.4	0.003
hard cheese [#]	0.2	0.0-0.9	0.038

* adjusted for age, gender, geography (north/south).

[#] smoked ham and hard cheese are confounders for cold cuts and Edamer cheese.

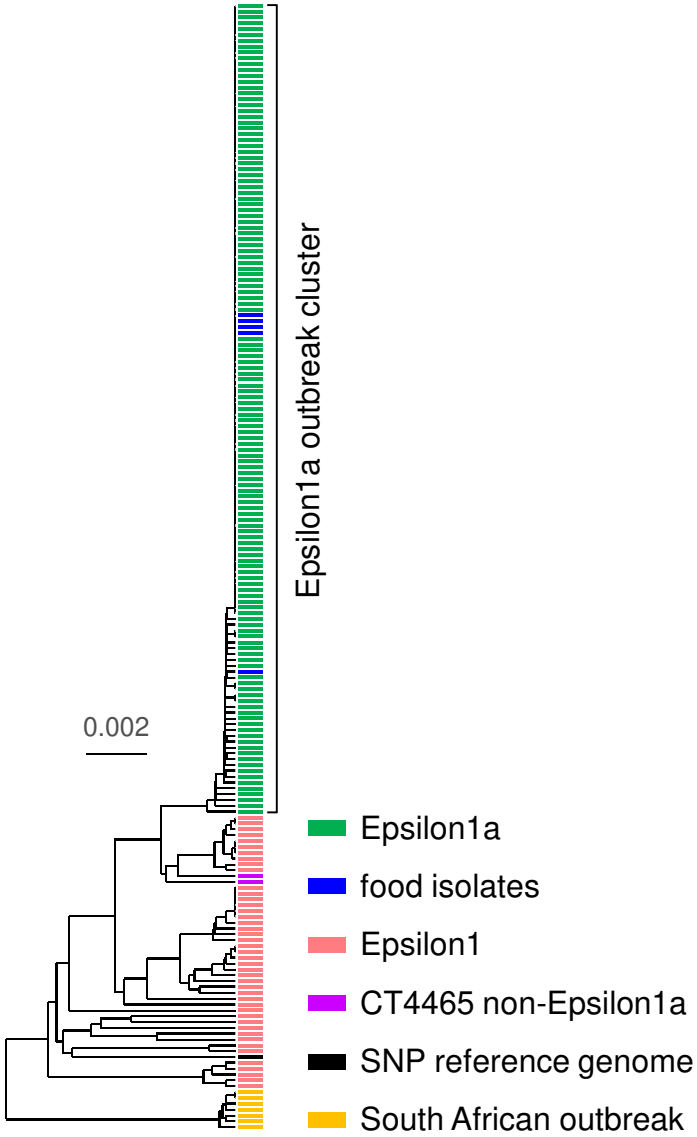
Epsilon1a outbreak cluster
0.002
Epsilon1a
food isolates
Epsilon1
CT4465 non-Epsilon1a
SNP reference genome
South African outbreak

Figure 1

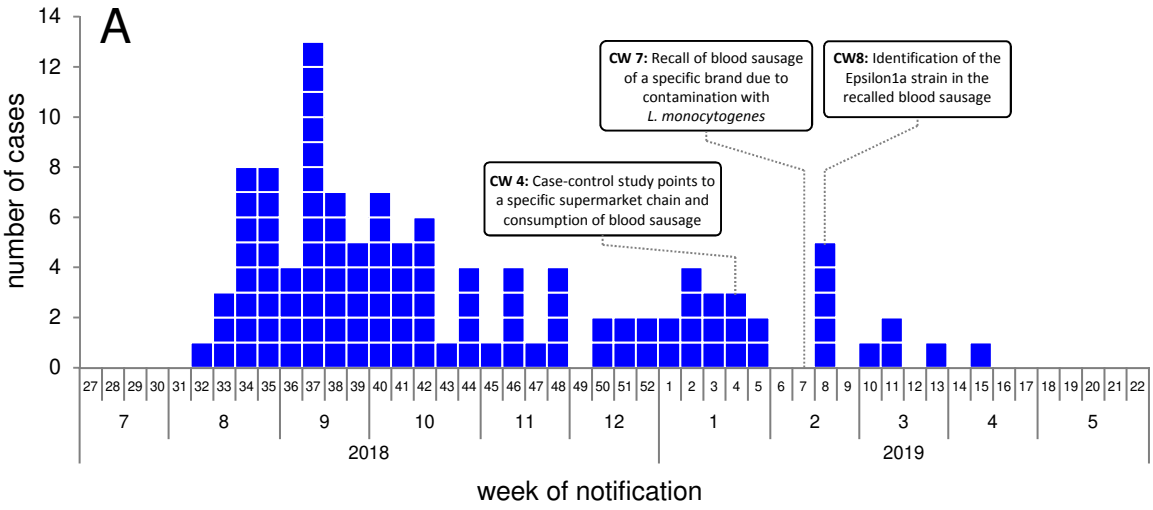
A
number of cases
0
2
4
6
8
10
12
14
CW 7: Recall of blood sausage of a specific brand due to contamination with L. monocytogenes
CW8: Identification of the Epsilon1a strain in the recalled blood sausage
CW 4: Case-control study points to a specific supermarket chain and consumption of blood sausage
27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
7 8 9 10 11 12 1 2 3 4 5
2018
2019
week of notification

Figure 2

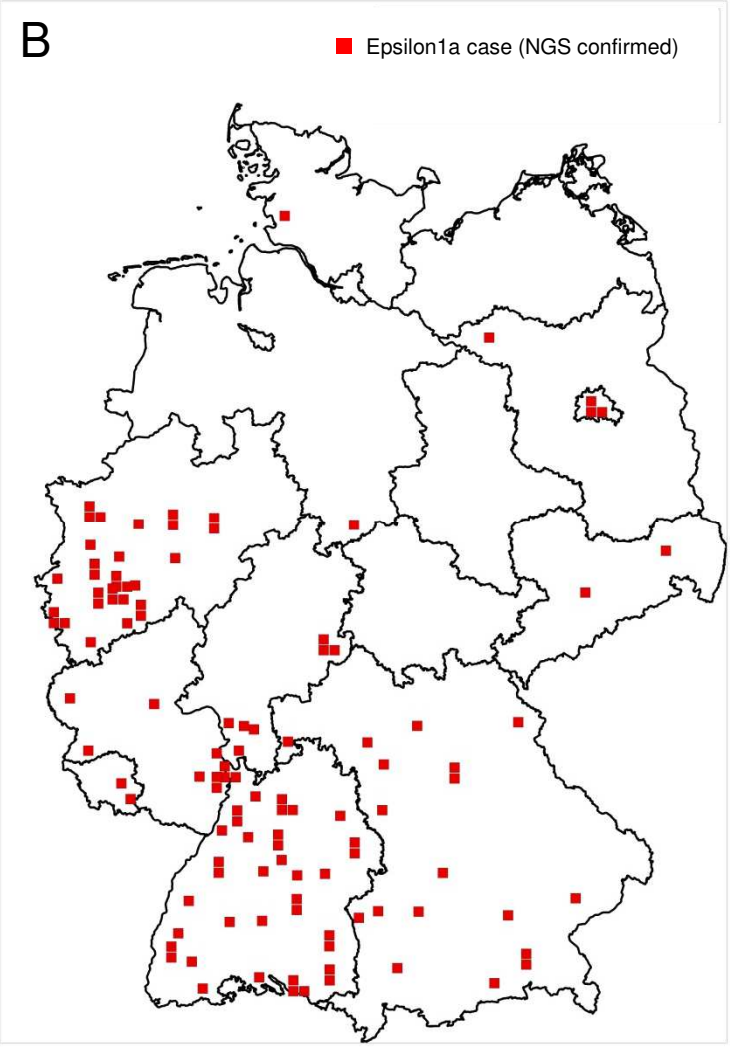

Figure 2

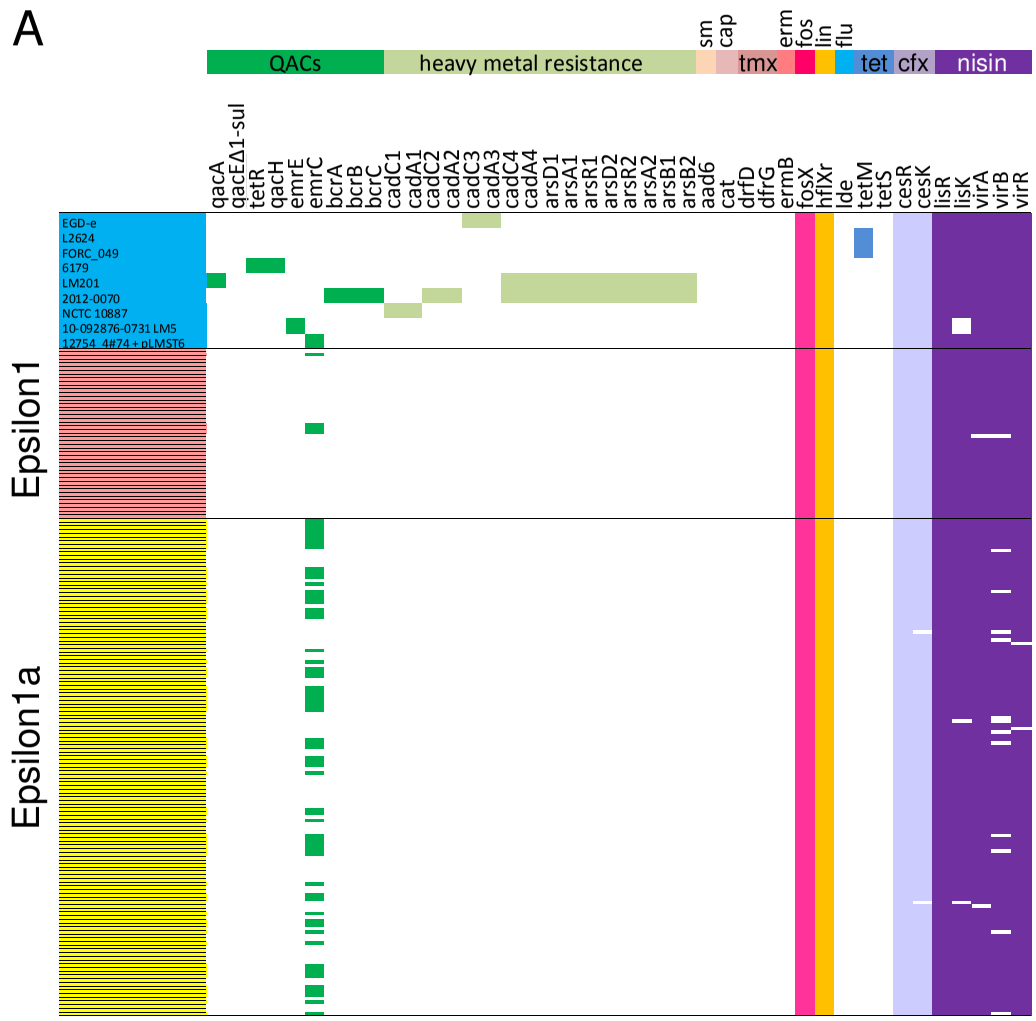
A
QACs
heavy metal resistance
sm
cap
tmx
erm
fos
lin
flu
tet
cfx
nisin
qacA
qacEΔ1-sul
tetR
qacH
emrE
emrC
bcrA
bcrB
bcrC
cadC1
cadA1
cadC2
cadA2
cadC3
cadA3
cadC4
cadA4
arsD1
arsA1
arsR1
arsD2
arsR2
arsA2
arsB1
arsB2
aad6
cat
drfD
dfrG
ermB
fosX
hflXr
lde
tetM
tetS
cesR
cesK
lisR
lisK
virA
virB
virR
EGD-e
L2624
FORC_049
6179
LM201
2012-0070
NCTC 10887
10-092876-0731 LM5
12754_4#74 + pLMST6
Epsilon1
Epsilon1a

Figure 3

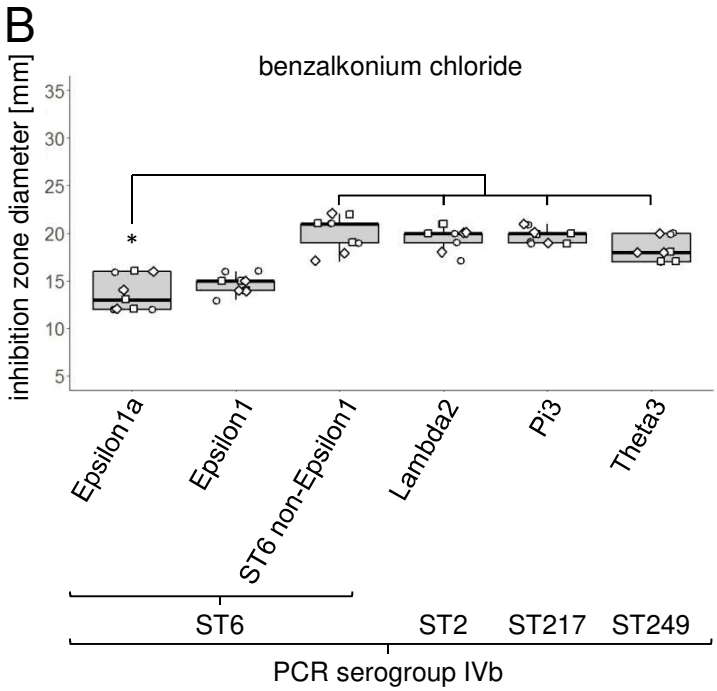
B
benzalkonium chloride
inhibition zone diameter [mm]
35
30
25
20
15
10
5
*
Epsilon1a
Epsilon1
ST6 non-Epsilon1
Lambda2
Pi3
Theta3
ST6
ST2
ST217
ST249
PCR serogroup IVb

Figure 3

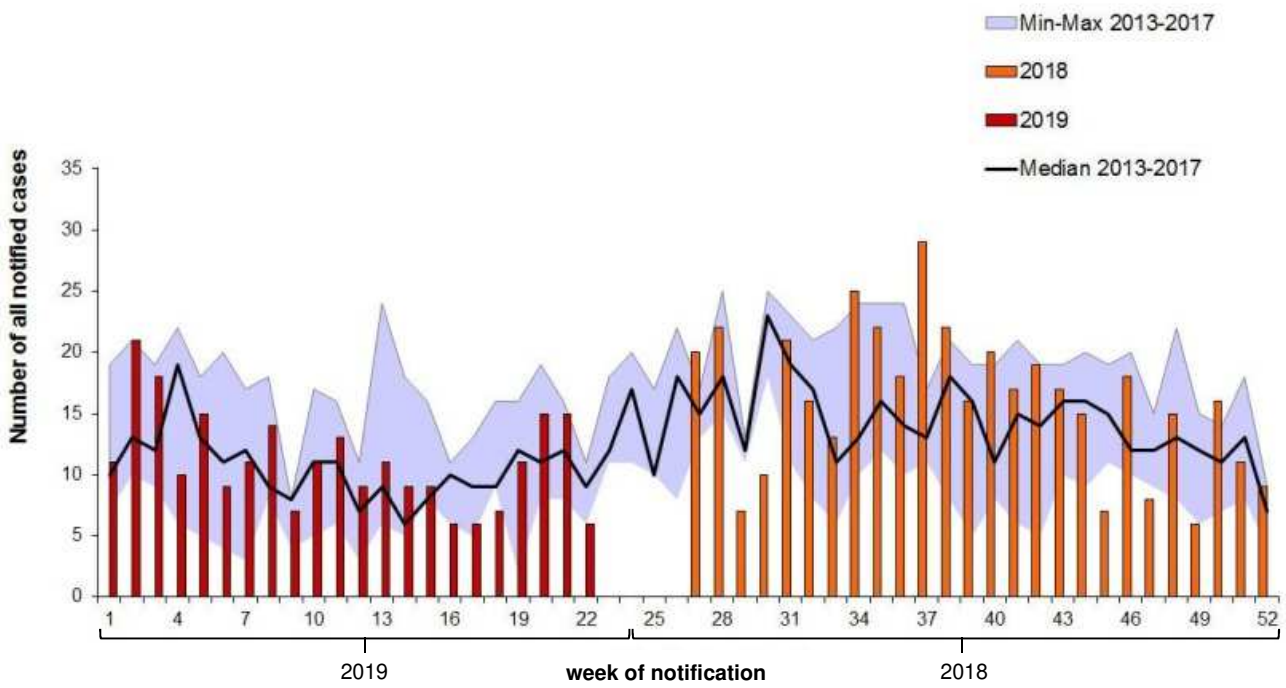

Figure S1: Weekly numbers of notified German listeriosis cases during the outbreak period in comparison to minimal, median and maximal case numbers reported per week in Germany during the reference period 2013-2017.

Figure S1

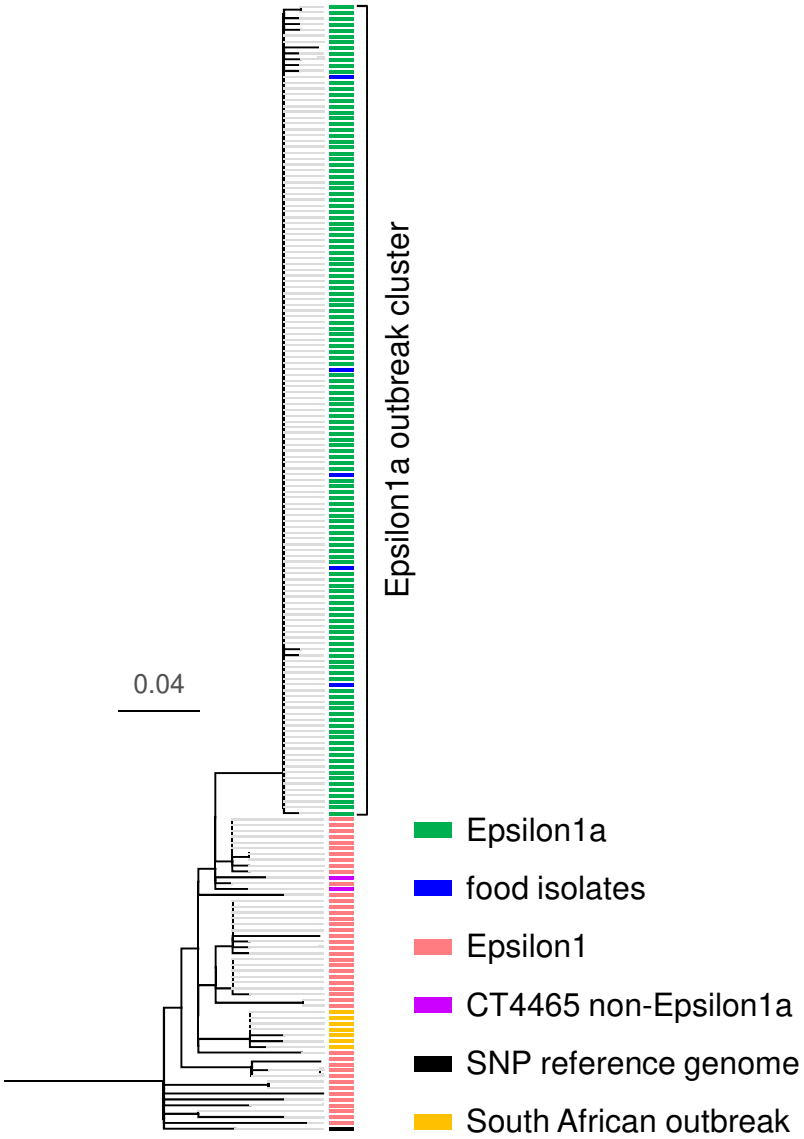

Figure S2: Confirmation of the Epsilon1a outbreak cluster by SNP calling.
Maximum likelihood tree illustrating phylogenetic relatedness of the same set of isolates as in Figure 1 after read mapping to the genome of the *L. monocytogenes* serogroup IVb strain 10-092876-0769 LM12 (39) as the reference and SNP filtering.

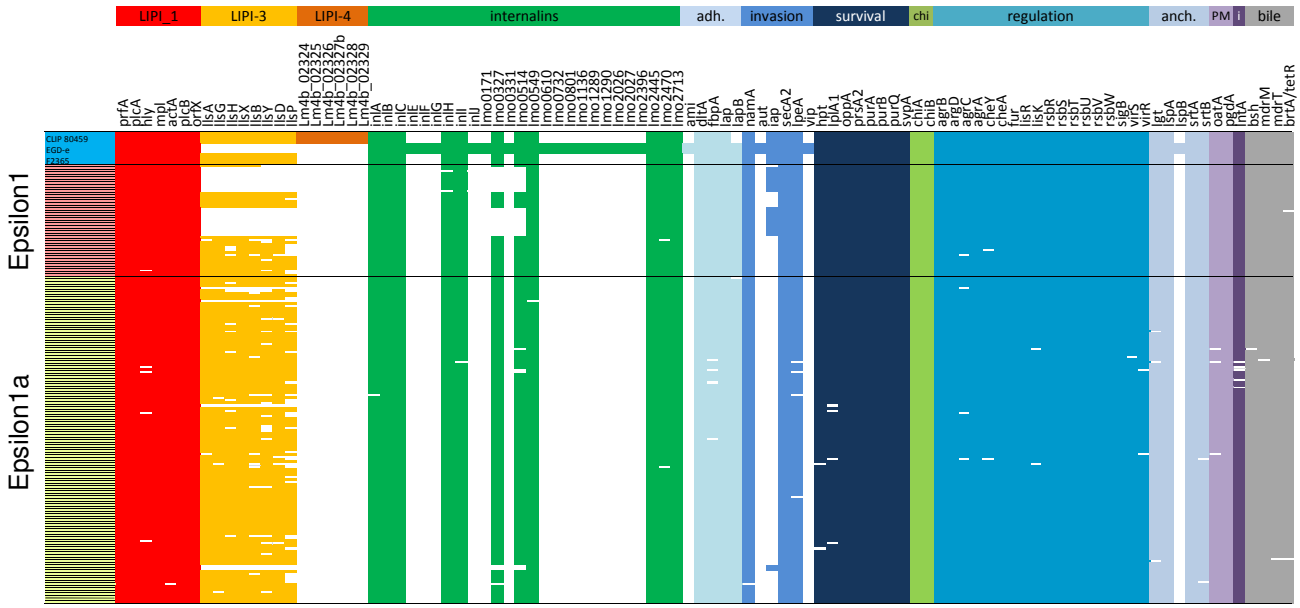

Figure S3: Virulome analysis of *L. monocytogenes* Epsilon1 and Epsilon1a isolates. Assembled genome sequences were searched for genes belonging to pathogenicity islands LIPI-1, LIPI-3 or LIPI-4, encoding internalins, or important for adhesion (adh.), invasion, intracellular survival, chitin hydrolysis (chi), gene regulation, protein anchoring to the cell surface (anch.), peptidoglycan modification (PM), immunomodulation (i) or bile resistance. Genomes of strains EGD-e (NC_003210.1), F2365 (NC_002973.6) and CLIP80459 (NC_012488.1) were included for comparison. The variability in the presence/absence of chromosomally encoded virulence genes within the highly clonal Epsilon1a cluster is likely not real and instead results from acceptance bias due to cut-off values used during allele calling.

Supplementary material for

Exceptionally large and country-wide outbreak of invasive listeriosis associated with blood sausage consumption, Germany 2018-2019

by

Sven Halbedel, Hendrik Wilking, Alexandra Holzer, Sylvia Kleta, Martin A. Fischer, Stefanie Lueth, Ariane Pietzka, Steliana Huhulescu, Raskit Lachmann, Amrei Krings, Werner Ruppitsch, Alexandre Leclerq, Rolf Kamphausen, Maylin Meincke, Christiane Wagner-Wiening, Matthias Contzen, Iris Barbara Kraemer, Sascha Al Dahouk, Franz Allerberger, Klaus Stark, Antje Flieger

Supplementary Methods: A diagnostic PCR for identification of the outbreak clone

Table S1: *L. monocytogenes* isolates included in this study.

Table S2: Antimicrobial susceptibility in the Epsilon1a outbreak cluster

A diagnostic PCR for identification of the outbreak clone

The median turn-around time (time from arrival of a sample in the consultant laboratory until genome sequencing results) was 34.5 days in the course of this investigation. Therefore, we searched for DNA regions specific for Epsilon1a isolates in order to identify Epsilon1a clones by PCR and to prioritize samples for genome sequencing and to initiate patient interviews without delay. For this purpose, the contigs of three Epsilon1a isolates (target) and of 50 non-Epsilon1a isolates of PCR serogroup IVb (non-target) were analyzed by the RUCS 1.0 algorithm, designed to identify primer pairs for unique core sequences present in a target genome dataset and absent in a set of non-target genomes. (1) This approach identified a 262 bp fragment specific for the chosen target genomes that could be amplified using the primers Eps-1-fw (AGTCGTCTTTAGTGCGCTGAA) and Eps-1-rev (TAGGTCTGTTGATGGCACCAC). The applicability of this primer set was tested using 16 genome sequences of Epsilon1a and 12 of non-Epsilon1a PCR serogroup IVb clones. Experimentally determined sensitivity of the PCR system was 94%, while its specificity was 75%. The 262 bp fragment is part of an open reading frame encoding a phage tail length tape measure protein, which was detected in 119 out of 134 Epsilon1a strains (89%) according to genomic data. In contrast, among 662 analyzed PCR serogroup IVb genomes, this phage tail open reading frame was only found in 20 ST6 genomes that did not belong to the Epsilon1a group (3%). Thus, the Eps1a PCR was used to identify possible Epsilon1a isolates among the incoming PCR serogroup IVb isolates to prioritize them for WGS. Out of the 67 PCR serogroup IVb isolates that had been tested by the Eps1a PCR during the outbreak, only 11 turned out to be false-positive (16%).

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Table S1: *L. monocytogenes* isolates included in this study

Isolate ID	Sample accession	Secondary accession	Study number	Source Type	Isolation source	Cluster	cgMLST complex type
11-04869	SAMEA104485064	ERS2103006	PRJEB24496	human	blood	Epsilon1	90
16-00332	SAMEA104485223	ERS2103165	PRJEB24496	human	blood	Epsilon1	90
16-00478	SAMEA104485231	ERS2103173	PRJEB24496	human	blood	Epsilon1	90
16-00634	SAMEA104485236	ERS2103178	PRJEB24496	human	CSF	Epsilon1	90
16-00830	SAMEA5770458	ERS3574002	PRJEB33238	human	blood	Epsilon1	90
16-00831	SAMEA104485244	ERS2103186	PRJEB24496	human	uterus	Epsilon1	90
16-00955	SAMEA104485248	ERS2103190	PRJEB24496	human	blood	Epsilon1	90
16-01401	SAMEA104485262	ERS2103204	PRJEB24496	human	blood	Epsilon1	90
16-01909	SAMEA104485285	ERS2103227	PRJEB24496	human	blood	Epsilon1	90
16-01911	SAMEA104485286	ERS2103228	PRJEB24496	human	blood	Epsilon1	90
16-02052	SAMEA104485291	ERS2103233	PRJEB24496	human	unknown	Epsilon1	3803
16-02281	SAMEA104485298	ERS2103240	PRJEB24496	human	blood	Epsilon1	3805
16-02328	SAMEA104485301	ERS2103243	PRJEB24496	human	unknown	Epsilon1	3806
16-02495	SAMEA104485307	ERS2103249	PRJEB24496	human	unknown	Epsilon1	90
16-02497	SAMEA104485309	ERS2103251	PRJEB24496	human	blood	Epsilon1	2981
16-02650	SAMEA104485313	ERS2103255	PRJEB24496	human	blood	Epsilon1	3921
16-03183	SAMEA104485347	ERS2103289	PRJEB24496	human	CSF	Epsilon1	4083
16-04063	SAMEA5770459	ERS3574003	PRJEB33238	human	blood	Epsilon1	90
16-04236	SAMEA104485396	ERS2103338	PRJEB24496	human	blood	Epsilon1	90
16-04386	SAMEA5770460	ERS3574004	PRJEB33238	human	blood	Epsilon1	90
16-04399	SAMEA104485408	ERS2103350	PRJEB24496	human	blood	Epsilon1	90
16-04799	SAMEA104485422	ERS2103364	PRJEB24496	human	ascites	Epsilon1	90
16-04800	SAMEA104485423	ERS2103365	PRJEB24496	human	blood	Epsilon1	90
16-05014	SAMEA104485430	ERS2103372	PRJEB24496	human	ascites	Epsilon1	90
17-00454	SAMEA104485458	ERS2103400	PRJEB24496	human	blood	Epsilon1	4083
17-00659	SAMEA5769034	ERS3572580	PRJEB33238	human	blood	Epsilon1	2981
17-01077	SAMEA5769035	ERS3572581	PRJEB33238	human	blood	Epsilon1	90
17-03140	SAMEA5769036	ERS3572582	PRJEB33238	human	blood	Epsilon1	4465
17-05508	SAMEA5769037	ERS3572583	PRJEB33238	human	blood	Epsilon1	2981
17-06068	SAMEA5769038	ERS3572584	PRJEB33238	human	blood	Epsilon1	90
17-06319	SAMEA5769039	ERS3572585	PRJEB33238	human	CSF	Epsilon1	90
17-06904	SAMEA5769040	ERS3572586	PRJEB33238	human	blood	Epsilon1	90
18-00080	SAMEA5769102	ERS3572648	PRJEB33238	human	CSF	Epsilon1	90
18-00304	SAMEA5769041	ERS3572587	PRJEB33238	human	blood	Epsilon1	6236
18-00445	SAMEA5769103	ERS3572649	PRJEB33238	human	unknown	Epsilon1	6331
18-01855	SAMEA5769042	ERS3572588	PRJEB33238	human	blood	Epsilon1	90
18-02683	SAMEA5769104	ERS3572650	PRJEB33238	human	blood	Epsilon1	4465
18-02987	SAMEA5769105	ERS3572651	PRJEB33238	human	GS	Epsilon1	90
18-03576	SAMEA5769106	ERS3572652	PRJEB33238	human	unknown	Epsilon1	2981
18-03577	SAMEA5769107	ERS3572653	PRJEB33238	human	GS	Epsilon1	2981
18-04116	SAMEA5769108	ERS3572654	PRJEB33238	human	blood	Epsilon1a	4465
18-04317	SAMEA5769109	ERS3572655	PRJEB33238	human	CSF	Epsilon1a	7353
18-04364	SAMEA5769110	ERS3572656	PRJEB33238	human	blood	Epsilon1a	4465
18-04365	SAMEA5769043	ERS3572589	PRJEB33238	human	blood	Epsilon1a	4465
18-04414	SAMEA5769111	ERS3572657	PRJEB33238	human	CSF	Epsilon1a	7353
18-04434	SAMEA5769112	ERS3572658	PRJEB33238	human	blood	Epsilon1a	4465

Isolate ID	Sample accession	Secondary accession	Study number	Source Type	Isolation source	Cluster	cgMLST complex type
18-04472	SAMEA5769113	ERS3572659	PRJEB33238	human	blood	Epsilon1	6331
18-04499	SAMEA5769114	ERS3572660	PRJEB33238	human	blood	Epsilon1a	4465
18-04500	SAMEA5769115	ERS3572661	PRJEB33238	human	blood	Epsilon1a	4465
18-04539	SAMEA5769116	ERS3572662	PRJEB33238	human	blood	Epsilon1a	4465
18-04540	SAMEA5041142	ERS2852884	PRJEB29295	human	blood	Epsilon1a	4465
18-04543	SAMEA5769044	ERS3572590	PRJEB33238	human	blood	Epsilon1a	4465
18-04581	SAMEA5769045	ERS3572591	PRJEB33238	human	blood	Epsilon1a	4465
18-04652	SAMEA5769046	ERS3572592	PRJEB33238	human	blood	Epsilon1a	4465
18-04653	SAMEA5769047	ERS3572593	PRJEB33238	human	blood	Epsilon1a	7353
18-04654	SAMEA5769048	ERS3572594	PRJEB33238	human	blood	Epsilon1a	4465
18-04655	SAMEA5769049	ERS3572595	PRJEB33238	human	blood	Epsilon1a	4465
18-04657	SAMEA5769050	ERS3572596	PRJEB33238	human	blood	Epsilon1a	7353
18-04772	SAMEA5769051	ERS3572597	PRJEB33238	human	blood	Epsilon1a	4465
18-04825	SAMEA5769052	ERS3572598	PRJEB33238	human	blood	Epsilon1a	7353
18-04826	SAMEA5769053	ERS3572599	PRJEB33238	human	blood	Epsilon1a	4465
18-04827	SAMEA5769117	ERS3572663	PRJEB33238	human	blood	Epsilon1a	7353
18-04850	SAMEA5769118	ERS3572664	PRJEB33238	human	blood	Epsilon1a	4465
18-04852	SAMEA5769119	ERS3572665	PRJEB33238	human	blood	Epsilon1a	4465
18-04897	SAMEA5769120	ERS3572666	PRJEB33238	human	blood	Epsilon1a	7353
18-04898	SAMEA5769121	ERS3572667	PRJEB33238	human	blood	Epsilon1a	4465
18-04954	SAMEA5769122	ERS3572668	PRJEB33238	human	blood	Epsilon1a	4465
18-04955	SAMEA5769123	ERS3572669	PRJEB33238	human	blood	Epsilon1a	4465
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18-05035	SAMEA5769125	ERS3572671	PRJEB33238	human	blood	Epsilon1a	7353
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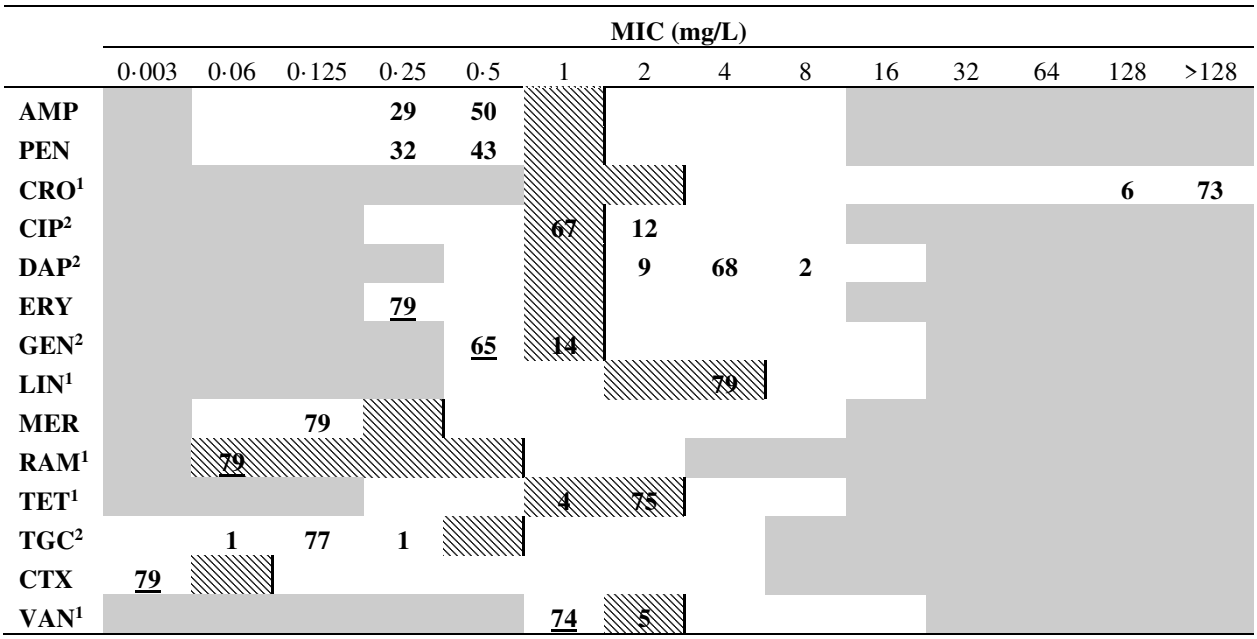
18-05084	SAMEA5769127	ERS3572673	PRJEB33238	human	blood	Epsilon1a	4465
18-05142	SAMEA5769128	ERS3572674	PRJEB33238	human	unknown	Epsilon1a	4465
18-05143	SAMEA5769129	ERS3572675	PRJEB33238	human	unknown	Epsilon1a	7353
18-05144	SAMEA5769130	ERS3572676	PRJEB33238	human	blood	Epsilon1a	4465
18-05199	SAMEA5769131	ERS3572677	PRJEB33238	human	blood	Epsilon1a	4465
18-05201	SAMEA5769132	ERS3572678	PRJEB33238	human	blood	Epsilon1a	4465
18-05202	SAMEA5769133	ERS3572679	PRJEB33238	human	blood	Epsilon1a	4465
18-05203	SAMEA5769134	ERS3572680	PRJEB33238	human	blood	Epsilon1a	4465
18-05237	SAMEA5769135	ERS3572681	PRJEB33238	human	blood	Epsilon1a	4465
18-05327	SAMEA5769136	ERS3572682	PRJEB33238	human	blood	Epsilon1a	4465
18-05328	SAMEA5769137	ERS3572683	PRJEB33238	human	blood	Epsilon1a	4465
18-05329	SAMEA5769138	ERS3572684	PRJEB33238	human	blood	Epsilon1a	4465
18-05393	SAMEA5769139	ERS3572685	PRJEB33238	human	CSF	Epsilon1a	4465
18-05394	SAMEA5769140	ERS3572686	PRJEB33238	human	blood	Epsilon1a	4465
18-05396	SAMEA5769141	ERS3572687	PRJEB33238	human	blood	Epsilon1a	7353
18-05398	SAMEA5769054	ERS3572600	PRJEB33238	human	blood	Epsilon1a	7353
18-05449	SAMEA5769142	ERS3572688	PRJEB33238	human	blood	Epsilon1a	4465
18-05450	SAMEA5769143	ERS3572689	PRJEB33238	human	blood	Epsilon1a	4465
18-05496	SAMEA5769144	ERS3572690	PRJEB33238	human	blood	Epsilon1a	4465
18-05542	SAMEA5769145	ERS3572691	PRJEB33238	human	CSF	Epsilon1a	4465
18-05544	SAMEA5769146	ERS3572692	PRJEB33238	human	blood	Epsilon1a	4465
18-05558	SAMEA5769147	ERS3572693	PRJEB33238	human	blood	Epsilon1a	4465
18-05655	SAMEA5769148	ERS3572694	PRJEB33238	human	blood	Epsilon1a	4465

Isolate ID	Sample accession	Secondary accession	Study number	Source Type	Isolation source	Cluster	cgMLST complex type
18-05657	SAMEA5769149	ERS3572695	PRJEB33238	human	CSF	Epsilon1a	4465
18-05658	SAMEA5769150	ERS3572696	PRJEB33238	human	blood	Epsilon1a	4465
18-05660	SAMEA5769151	ERS3572697	PRJEB33238	human	blood	Epsilon1a	4465
18-05714	SAMEA5769152	ERS3572698	PRJEB33238	human	blood	Epsilon1a	4465
18-05726	SAMEA5769153	ERS3572699	PRJEB33238	human	blood	Epsilon1a	7353
18-05748	SAMEA5769154	ERS3572700	PRJEB33238	human	blood	Epsilon1a	4465
18-05767	SAMEA5769155	ERS3572701	PRJEB33238	human	blood	Epsilon1a	4465
18-05768	SAMEA5769156	ERS3572702	PRJEB33238	human	CSF	Epsilon1a	4465
18-05836	SAMEA5769157	ERS3572703	PRJEB33238	human	unknown	Epsilon1a	7353
18-05837	SAMEA5769055	ERS3572601	PRJEB33238	human	blood	Epsilon1a	7353
18-05951	SAMEA5769158	ERS3572704	PRJEB33238	human	blood	Epsilon1a	4465
18-05970	SAMEA5769159	ERS3572705	PRJEB33238	human	CSF	Epsilon1a	4465
18-06023	SAMEA5769056	ERS3572602	PRJEB33238	human	unknown	Epsilon1a	7353
18-06024	SAMEA5769057	ERS3572603	PRJEB33238	human	ascites	Epsilon1a	4465
18-06035	SAMEA5769058	ERS3572604	PRJEB33238	human	blood	Epsilon1a	4465
18-06036	SAMEA5769059	ERS3572605	PRJEB33238	human	blood	Epsilon1a	4465
18-06121	SAMEA5769060	ERS3572606	PRJEB33238	human	blood	Epsilon1a	4465
18-06126	SAMEA5769061	ERS3572607	PRJEB33238	human	blood	Epsilon1a	4465
18-06127	SAMEA5769062	ERS3572608	PRJEB33238	human	CSF	Epsilon1a	4465
18-06128	SAMEA5769063	ERS3572609	PRJEB33238	human	blood	Epsilon1a	4465
18-06129	SAMEA5769064	ERS3572610	PRJEB33238	human	blood	Epsilon1a	4465
18-06130	SAMEA5769065	ERS3572611	PRJEB33238	human	blood	Epsilon1a	7353
18-06131	SAMEA5769066	ERS3572612	PRJEB33238	human	CSF	Epsilon1a	4465
18-06138	SAMEA5769067	ERS3572613	PRJEB33238	human	blood	Epsilon1a	4465
18-06170	SAMEA5769160	ERS3572706	PRJEB33238	human	unknown	Epsilon1a	4465
18-06263	SAMEA5769161	ERS3572707	PRJEB33238	human	blood	Epsilon1a	4465
18-06331	SAMEA5769162	ERS3572708	PRJEB33238	human	blood	Epsilon1a	4465
18-06438	SAMEA5769163	ERS3572709	PRJEB33238	human	unknown	Epsilon1a	4465
18-06540	SAMEA5769164	ERS3572710	PRJEB33238	human	blood	Epsilon1a	4465
18-06541	SAMEA5769165	ERS3572711	PRJEB33238	human	CSF	Epsilon1a	4465
18-06646	SAMEA5769166	ERS3572712	PRJEB33238	human	CSF	Epsilon1a	4465
18-06680	SAMEA5769167	ERS3572713	PRJEB33238	human	blood	Epsilon1a	4465
18-06776	SAMEA5769168	ERS3572714	PRJEB33238	human	blood	Epsilon1a	4465
18-06820	SAMEA5769169	ERS3572715	PRJEB33238	human	unknown	Epsilon1a	7353
18-06916	SAMEA5769170	ERS3572716	PRJEB33238	human	blood	Epsilon1a	4465
18-06954	SAMEA5769068	ERS3572614	PRJEB33238	human	blood	Epsilon1a	7353
18-06955	SAMEA5769069	ERS3572615	PRJEB33238	human	blood	Epsilon1a	4465
18-07018	SAMEA5769070	ERS3572616	PRJEB33238	human	blood	Epsilon1a	4465
18-07037	SAMEA5769071	ERS3572617	PRJEB33238	human	blood	Epsilon1a	4465
18-07092	SAMEA5769072	ERS3572618	PRJEB33238	human	unknown	Epsilon1a	4465
18-07157	SAMEA5769073	ERS3572619	PRJEB33238	human	blood	Epsilon1a	4465
18-07158	SAMEA5769074	ERS3572620	PRJEB33238	human	blood	Epsilon1a	4465
18-07267	SAMEA5769171	ERS3572717	PRJEB33238	human	blood	Epsilon1a	4465
18-07300	SAMEA5769172	ERS3572718	PRJEB33238	human	unknown	Epsilon1a	7353
19-00076	SAMEA5769173	ERS3572719	PRJEB33238	human	blood	Epsilon1a	4465
19-00080	SAMEA5769174	ERS3572720	PRJEB33238	human	blood	Epsilon1	90
19-00082	SAMEA5769175	ERS3572721	PRJEB33238	human	unknown	Epsilon1a	4465
19-00149	SAMEA5769176	ERS3572722	PRJEB33238	human	CSF	Epsilon1a	4465

Isolate ID	Sample accession	Secondary accession	Study number	Source Type	Isolation source	Cluster	cgMLST complex type
19-00151	SAMEA5769177	ERS3572723	PRJEB33238	human	blood	Epsilon1a	4465
19-00179	SAMEA5769178	ERS3572724	PRJEB33238	human	unknown	Epsilon1a	4465
19-00191	SAMEA5769179	ERS3572725	PRJEB33238	human	blood	Epsilon1a	4465
19-00240	SAMEA5769180	ERS3572726	PRJEB33238	human	unknown	Epsilon1a	4465
19-00278	SAMEA5769181	ERS3572727	PRJEB33238	human	blood	Epsilon1a	4465
19-00281	SAMEA5769182	ERS3572728	PRJEB33238	human	blood	Epsilon1a	7353
19-00312	SAMEA5769075	ERS3572621	PRJEB33238	human	blood	Epsilon1a	7353
19-00347	SAMEA5769076	ERS3572622	PRJEB33238	human	blood	Epsilon1a	4465
19-00419	SAMEA5769077	ERS3572623	PRJEB33238	human	blood	Epsilon1a	4465
19-00444	SAMEA5769078	ERS3572624	PRJEB33238	human	blood	Epsilon1a	4465
19-00499	SAMEA5769079	ERS3572625	PRJEB33238	human	blood	Epsilon1a	4465
19-00500	SAMEA5769080	ERS3572626	PRJEB33238	human	blood	Epsilon1a	4465
19-00520	SAMEA5769081	ERS3572627	PRJEB33238	human	blood	Epsilon1a	4465
19-00549	SAMEA5769082	ERS3572628	PRJEB33238	human	blood	Epsilon1a	4465
19-00582	SAMEA5769083	ERS3572629	PRJEB33238	human	blood	Epsilon1a	7353
19-00609	SAMEA5769084	ERS3572630	PRJEB33238	human	unknown	Epsilon1a	7353
19-00973	SAMEA5769183	ERS3572729	PRJEB33238	human	unknown	Epsilon1a	4465
19-00974	SAMEA5769184	ERS3572730	PRJEB33238	human	blood	Epsilon1a	7353
19-00998	SAMEA5769185	ERS3572731	PRJEB33238	human	blood	Epsilon1a	4465
19-01023	SAMEA5769186	ERS3572732	PRJEB33238	human	blood	Epsilon1a	4465
19-01108	SAMEA5769187	ERS3572733	PRJEB33238	human	blood	Epsilon1a	4465
19-01166	SAMEA5769188	ERS3572734	PRJEB33238	human	placenta	Epsilon1	90
19-01173	SAMEA5769189	ERS3572735	PRJEB33238	human	blood	Epsilon1	90
19-01197	SAMEA5769190	ERS3572736	PRJEB33238	human	blood	Epsilon1a	4465
19-01319	SAMEA5769191	ERS3572737	PRJEB33238	human	synovia	Epsilon1a	4465
19-01387	SAMEA5769192	ERS3572738	PRJEB33238	human	PF	Epsilon1a	4465
19-01604	SAMEA5769193	ERS3572739	PRJEB33238	human	wound	Epsilon1a	4465
19-01607	SAMEA5769194	ERS3572740	PRJEB33238	human	BA	Epsilon1a	4465
19-01930	SAMEA5769195	ERS3572741	PRJEB33238	human	blood	Epsilon1a	4465
19-01961	SAMEA5769196	ERS3572742	PRJEB33238	human	blood	Epsilon1	90
19-02578	SAMEA5769085	ERS3572631	PRJEB33238	human	blood	Epsilon1a	7353
19-02579	SAMEA5769086	ERS3572632	PRJEB33238	human	blood	Epsilon1a	7353
19-02581	SAMEA5769087	ERS3572633	PRJEB33238	human	blood	Epsilon1a	4465
19-02587	SAMEA5769088	ERS3572634	PRJEB33238	human	blood	Epsilon1a	7353
19-02590	SAMEA5769089	ERS3572635	PRJEB33238	human	CSF	Epsilon1a	4465
19-02598	SAMEA5769090	ERS3572636	PRJEB33238	human	CSF	Epsilon1a	4465
19-02600	SAMEA5769091	ERS3572637	PRJEB33238	human	blood	Epsilon1a	4465
19-LI00135-0	SAMEA5769092	ERS3572638	PRJEB33238	food		Epsilon1a	4465
19-LI00136-0	SAMEA5769093	ERS3572639	PRJEB33238	food		Epsilon1a	4465
19-LI00137-0	SAMEA5769094	ERS3572640	PRJEB33238	food		Epsilon1a	4465
19-LI00138-0	SAMEA5769095	ERS3572641	PRJEB33238	food		Epsilon1a	4465
19-LI00175-0	SAMEA5769096	ERS3572642	PRJEB33238	food		Epsilon1a	4465

Abbreviations: CSF - cerebrospinal fluid, GS - gynaecological swab, PF - pleural fluid, BA - brain abscess. Please not that isolates from non-sterile materials were not included in the outbreak description.

Table S2: Antimicrobial susceptibility in the Epsilon1a outbreak cluster (n= 79).



All 79 strains were tested against 14 antibiotics. Underlined values indicate no observable growth at the lowest tested concentration. Concentrations in grey areas were not tested. Vertical lines indicate resistance breakpoints as defined by EUCAST for *Listeria monocytogenes*, *Streptococcus pneumoniae* (1) or *Staphylococcus aureus* (2). Intermediate concentration values between resistant and susceptible organisms are hatched.

Abbreviations: AMP - ampicillin, PEN - benzylpenicillin, CRO - ceftriaxone, CIP - ciprofloxacin, DAP - daptomycin, ERY - erythromycin, GEN - gentamicin, LIN - linezolid, MER - meropenem, RAM - rifampicin, TET - tetracycline, TGC - tigecycline, CTX - cotrimoxazole (trimethoprim/ sulfamethoxazole), VAN - vancomycin.