

# Biochemical and molecular characteristics of *Listeria monocytogenes* isolates from a prosthetic mitral heart valve-bearing patient's blood cultures

## Características bioquímicas e moleculares de *Listeria monocytogenes* isolados de hemoculturas de próteses de válvula mitral

Ana Paula Rocha da Costa<sup>1,3</sup>, Marinalda Anselmo Vilela<sup>2</sup>, Carina Lucena Mendes-Marques<sup>3</sup>, Alzira Maria Paiva de Almeida<sup>3</sup>, Nilma Cintra Leal<sup>3</sup>

1. Departamento de Ciências Biológicas, Universidade Federal de Pernambuco, Recife, PE, Brasil. 2. Laboratório de Resistência Microbiana, Instituto de Ciências Biológicas, Universidade de Pernambuco, Recife, PE, Brasil. 3. Centro de Pesquisas Aggeu Magalhães, FIOCRUZ-PE, Recife, PE, Brasil.

### Abstract

**Background:** In Brazil, listeriosis is not a notifiable disease; thus, the incidence of Brazilian cases remains unknown. *Listeria monocytogenes* is not always included in automated systems, and its detection depends on the high skill level of microbiology laboratory professionals. This paper describes the characteristics of *L. monocytogenes* isolates fortuitously obtained from an endocarditis case in Recife, PE, Brazil. **Methods:** Six bacterial isolates obtained from six blood cultures from a 28-year-old male bearing a prosthetic mitral heart valve were analyzed by PCR using primers specific of *L. monocytogenes* to confirm a presumptive identification, determine the serotype and presence of the virulence genes (*inlA*, *inlB*, *inlC*, *inlJ*, *hly*, *plcA*, *actA*, *prfA*) in an attempt to determine the *Listeria* genotype by PCR-ribotyping. **Results:** The samples were identified as *L. monocytogenes* 4b. All investigated virulence genes were amplified by PCR, and the identity of the amplified segments was confirmed by sequencing. A deletion of 105 base pairs was detected in the *actA* gene. All of the samples generated the same PCR-ribotype pattern, clustered into a single ribotype, and were considered a single strain. **Conclusion:** *L. monocytogenes* infection should be considered in endocarditis differential diagnoses, especially among high-risk groups, due to its high pathogenicity and the environmental ubiquity.

**Keywords:** *Listeria*. Listeriosis. *Listeria monocytogenes*. Endocarditis. Virulence.

### Resumo

**Introdução:** A listeriose não é uma doença de notificação obrigatória no Brasil e relatos de casos clínicos são escassos. *Listeria monocytogenes* não é rotineiramente incluída nos sistemas automatizados e sua detecção depende da habilidade dos profissionais dos laboratórios de microbiologia. Neste trabalho, descrevemos as características de isolados de *L. monocytogenes* obtidos de um caso de endocardite em Recife, PE, Brasil. **Métodos:** Seis isolados de seis hemoculturas obtidas de um paciente de 28 anos, portador de válvula cardíaca mitral protética foram analisados por PCR utilizando primers específicos para confirmar a identificação, determinar o sorotipo e a presença dos genes de virulência (*inlA*, *inlB*, *inlC*, *inlJ*, *hly*, *plcA*, *actA*, *prfA*) e para determinar o genótipo dos isolados por PCR-ribotipagem. **Resultados:** As amostras foram identificadas como *L. monocytogenes* 4b. Todos os genes de virulência investigados foram amplificados, e a identidade dos segmentos foi confirmada por sequenciamento. Uma deleção de 105 pares de bases foi detectada no gene *actA*. Todas as amostras revelaram padrão de amplificação similar por PCR-ribotipagem e foram consideradas uma única estirpe. **Conclusão:** Infecção por *L. monocytogenes* deve ser considerada em diagnósticos diferenciais de endocardite, especialmente entre os grupos de alto risco, devido à sua alta patogenicidade e ubiquidade ambiental.

**Palavras-chave:** *Listeria*. Listeriose. *Listeria monocytogenes*. Endocardite. Virulência.

### BACKGROUND

*Listeria monocytogenes* is a rod-shaped gram-positive facultative intracellular pathogen that causes the disease so called listeriosis. It primarily affects pregnant women, newborns and immunocompromised adults, causing sepsis, abortion and infection of the central nervous system and resulting in high morbidity and mortality<sup>1</sup>. Out of the 13 recognized *L. monocytogenes* serotypes, three (1/2a, 1/2b and 4b) appear to be more pathogenic; they are

epidemiologically associated with the most serious cases of human listeriosis<sup>2</sup>.

In general, cardiovascular diseases, such as endocarditis and pericarditis, are associated with a poor prognosis and high mortality<sup>3,4,5</sup>. Endocarditis caused by *L. monocytogenes* infection often occurs in patients with defective or prosthetic heart valves, especially aortic and mitral valves<sup>6,7</sup>. Mortality

**Correspondence:** nilma@cpqam.fiocruz.br

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occurs in 100% of untreated cases; however, appropriate treatment reduces this rate to 20-30%. This mortality rate is still higher than for endocarditis caused by another bacteria, which is typically 14% lethal<sup>4</sup>. *L. monocytogenes* endocarditis is clinically diagnosed by symptoms associated with bacteremia, and treatment of infections is usually accomplished through the administration of antibiotics, often ampicillin and an aminoglycoside<sup>3</sup>. Early identification of *L. monocytogenes* in endocarditis cases and the determination of its antimicrobial sensitivity are crucial for the treatment of affected patients. However, *Listeria* is seldom suspected as the causative agent of endocarditis, and patient treatment is typically tailored for bacteria that are more frequently involved in endocarditis such as *Staphylococcus aureus*, *Streptococcus viridans*, *Enterococcus* and coagulase negative *Staphylococcus* (CoNS)<sup>8</sup>. Therefore, the establishment of appropriate treatments may be delayed, aggravating the patient's condition and possibly leading to death as a result of the high pathogenicity of *L. monocytogenes*<sup>9</sup>.

This study analyzed six *L. monocytogenes* cultures as obtained from a prosthetic mitral heart valve-bearing patient who presented it at an emergency cardiology facility. The presumptive *Listeria* identification was confirmed by PCR at the genus, species and serotype levels. The genomic profile of the organism was analyzed by PCR-ribotyping, *Listeria* virulence genes involved in the infection pathway of the main host cells were detected, and the antimicrobial susceptibility of the strains was assessed.

## METHODS

### Bacteria isolation, presumptive identification and antimicrobial susceptibility assessment

Six blood cultures were obtained from a 28-year-old male bearing a bovine pericardial mitral heart valve who presented himself in a cardiology emergency facility in Recife, PE, Brazil. Following clinical evaluation, three blood cultures were collected at 30-minute intervals; however, endocarditis or *Listeria* infection was not suspected, and the patient was released before the blood culture results were completed. Four days later, the patient entered the intensive care unit after his condition had deteriorated. A second series of blood cultures was collected, and the patient was successfully diagnosed and treated, resulting in a full recovery.

The bacterial growth after 24 hours of incubation was initially identified as *Listeria* spp. by routine screening using standard bacteriological tests (colony morphology on blood agar plates, Gram staining and motility in semisolid medium)<sup>10</sup>. Antimicrobial susceptibility was determined by disk diffusion on agar plates according to CLSI guidelines for *Staphylococcus* spp.<sup>11</sup> and Soussy et al. (1994)<sup>12</sup> for ampicillin and vancomycin.

Four reference strains from the Collection of the Pasteur Institute (CLIP), the Centers for Disease Control (CDC), and the American Type Culture Collection were used: CLIP12612 (*L. innocua*, negative control), CDC F4561 (*L. monocytogenes* 1/2a), CDC F4976 (*L. monocytogenes* 1/2b), and ATCC 19115 (*L. monocytogenes* 4b, positive control) were accounted for.

### DNA extraction

DNA samples were extracted following a protocol based on Ausubel et al.<sup>13</sup>, with minor modifications. Briefly, 1 ml of each bacterial culture in brain heart infusion broth (BHI, Biobras) was centrifuged for 10 minutes at 14,000 rpm, the resulting pellet was washed with 500 µL of Tris:EDTA (10:1), and 10 µL of 1% lysozyme and 10 µL of 0.5% Proteinase K were added. The samples were incubated at 60°C for 20 minutes, then 100 µL of STE (2.5% SDS, 0.25 M EDTA, 10 mM Tris pH 8.0) was added. The samples were then incubated at 60°C for 15 minutes, at room temperature for 5 minutes and in an ice bath for 5 minutes. Next, 130 µL of 7.5 M sodium acetate was added, and the samples were re-incubated in an ice bath for 15 minutes and centrifuged for 3 minutes at 14,000 rpm. At this point, 700 µL of the supernatant was transferred to a new tube, 420 µL of isopropanol was added, and the samples were then incubated at -80°C for 30 minutes and centrifuged at 14,000 rpm for 10 minutes. The supernatant was discarded, and the pellet DNA was vacuum dried and resuspended in 10 µL of 0.02% RNase. DNA yield was quantified by comparison with known amounts of λ *Hind*III DNA (Sigma).

### PCR identification: genus, species and serotyping

To confirm the identification of the culture, specific primers were used in PCR reactions for amplification of the 23S rRNA gene from the *Listeria* genus<sup>14</sup> and *lmo* 2243 from *L. monocytogenes* species<sup>15</sup>. The serotype was assessed using primers specifically directed to genes from the three most pathogenic serotypes: *lmo* 0737 for 1/2a<sup>15</sup>, *gltB* for 1/2b<sup>16</sup> and ORF2110 for 4b<sup>17</sup>. The reaction mixtures were prepared in 25 µL volume and included 20 ng of genomic DNA, 50 mM KCl, 10 mM TrisHCl (pH 8.0), 200 mM dNTP (Invitrogen), 15 mM MgCl<sub>2</sub>, 20 pM of each primer and 1U of *Taq* DNA polymerase (Promega). Amplifications were performed in a Biometra thermocycler programmed for 30 cycles of 1 min at 92°C, 1 min at 55°C and 1 min at 72°C followed by a 7 min final extension at 72°C. As a negative control, a tube containing all reagents but genomic DNA was used in each reaction.

### Detection of virulence genes

The presence of virulence genes involved in the three stages of infection was assessed by PCR under the conditions

described above. Specific primers were used to detect *inlA*<sup>18</sup>, *inlB*<sup>19</sup>, *inlC*<sup>20</sup> and *inlJ*<sup>20</sup>, which are involved in host cell adhesion and invasion; *hly*<sup>14</sup> and *plcA*<sup>21</sup>, which are involved in vacuole escape; *actA*<sup>22</sup>, which is involved in intracellular propagation; and *prfA*<sup>21</sup>, which acts as a virulence gene regulator.

Each amplified gene segment was purified and sequenced in an automated ABI3100 sequencer (Applied Biosystems, USA). The consensus sequence for each gene was compared with published sequences available in GenBank (<http://www.ncbi.nlm.nih.gov>) using the Basic Local Alignment Tool (BLASTn). Multiple alignments were performed by Mega<sup>423</sup>.

### PCR-ribotyping

Ribotyping was performed by PCR using specific primers for amplification of the 16S-23S rDNA intergenic spacer region as described<sup>24</sup>.

## RESULTS

### Antimicrobial susceptibility

All samples revealed sensitivity to ampicillin, cephalothin, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, penicillin, rifampin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin and resistance to cefoxitin by agar disk diffusion.

### Determination of the genus, species and serotype by PCR

Segments of the expected size for the 23S rRNA gene specific to the *Listeria* genus (239 base pairs = bp), the *lmo2234* gene specific to *L. monocytogenes* species (420 bp) and the ORF2110 gene specific to serotype 4b (597 bp) were amplified in all samples (data not shown), and the samples were thus determined to be *L. monocytogenes* serotype 4b. *lmo 0737* for 1/2a and *gltB* for 1/2b did not amplify.

### Virulence genes

Segments of the expected size were amplified in all samples for the *inlA*, *inlB*, *inlC* and *inlJ* genes, which are involved in the bacterium adhesion and host cells invasion; the *hly* and *plcA* genes, which are involved in vacuole escape; and the *prfA* gene, which regulates virulence genes, (data not shown). Comparisons of the amplified segment sequences with published sequences revealed 98 to 100% identity (data not shown).

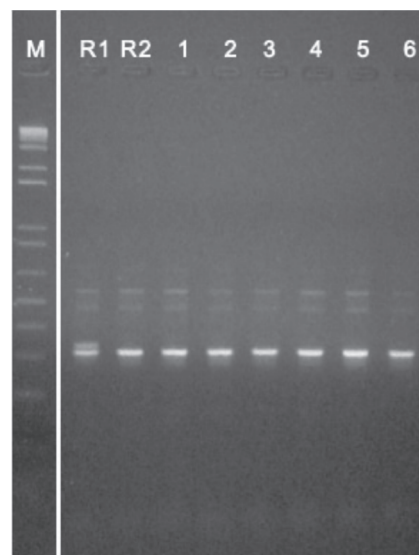
A 432 bp segment was detected instead of the expected 537 bp segment, indicating a 105 bp deletion. This amplicon was present in all samples for the *actA* gene, which is involved in the ability to spread cell-to-cell (Figure 1).



**Figure 1.** Multiple alignments of partial sequences showing the 105 bp deletion in the *actA* gene in samples from blood cultures compared with reference strain sequences.

### PCR-ribotyping

All samples generated a similar amplification pattern of the 16S-23S rDNA intergenic spacer region and therefore clustered into the same ribotype. Control serotypes 1/2a and 1/2b displayed a similar amplification pattern, clustering into the same ribotype (named R1), while serotype 4b generated a different amplification pattern and therefore clustered in a separate ribotype (named R2). The six blood culture samples clustered into ribotype 2 (Figure 2).



**Figure 2.** PCR ribotyping profile. Lines: M: 100bp Molecular marker; R1: *Listeria monocytogenes* ATCC 19155, serotype 4b; R2: *Listeria monocytogenes* CDC F4976, serotype 1/2b; 1-6: samples from the study.

## DISCUSSION

*L. monocytogenes* can be misidentified by gram-stain-determined morphology as *Corynebacterium* spp, which is often regarded as a contaminant, especially when the colonies grown from the clinical sample are scarce. In our region, *L. monocytogenes* is not always included in automated systems and is not often an expected cause of infection because of its low clinical frequency. Therefore, its detection depends on a high level of skill among microbiology laboratory professionals. In Brazil, listeriosis is not a notifiable disease, so the number of Brazilian cases remains unknown<sup>25</sup>. However, the severity of this life-threatening disease demands rapid treatment to avoid a fatal outcome.

Most clinical *L. monocytogenes* isolates are susceptible to a wide range of antibiotics<sup>26,27</sup>; however, resistance to chloramphenicol, erythromycin, streptomycin, tetracycline, vancomycin and trimethoprim has been reported<sup>28</sup>. In our region, the various automated identification systems widely employed in clinical microbiology laboratories, although easy and quick, they are not always programmed for low-frequency organisms like *L. monocytogenes*. The need for accurate antimicrobial susceptibility determination of the microorganisms from clinical material is crucial to avoid ineffective drug use, which can slow the treatment process, aggravate the patient's clinical condition, and possibly lead to a fatal outcome. In this study, antimicrobial susceptibility tests were carried out through disk diffusion on agar, and the cultures were resistant only to cefoxitin.

The six cultures were classified as serotype 4b, the most frequent causative agent of human listeriosis, and clustered into the same ribotype, indicating a single strain. All of the cultures harbored the virulence genes involved in the different stages of the infection. Surprisingly, the *actA* gene, which is involved in actin-dependent intracellular motility, displayed a 105 bp deletion. Others have reported a decrease in lethality in mice and cell-to-cell spreading in plaque formation

assays of serotype 4b strains carrying a similar deletion<sup>29</sup>. A substitution of alanine for proline at position 267 in the *actA* gene, or the absence of other genes, such as *inlC* and *inlJ*, may be responsible for the reduced intracellular propagation capacity<sup>30</sup>. However, the ability to spread to other cells and lethality in mice were not significantly reduced by the same 105 bp deletion in the *actA* gene in serotype 1/2b<sup>31</sup>. In our study, in spite of the 105 bp deletion in the *actA* gene, the strain remained pathogenic and capable of invading the blood stream. It is possible that other virulence-related genes are involved, or that the *actA* gene is not needed for infection in highly susceptible individuals.

*L. monocytogenes* endocarditis is rather infrequent, with barely over 70 cases published in the world<sup>32</sup>. Individuals with a history of rheumatic heart disease, hypertrophic cardiomyopathy, mitral valve prolapse or ischemic cardiomyopathy are particularly susceptible<sup>4,32</sup>. The average age of *L. monocytogenes* endocarditis patients increased from 47.1 years during the period from 1955 to 1984 to 65.5 years from 1985 to 2000<sup>4</sup>. The patient from our study was the youngest (28 years) among those patients published literature available.

## CONCLUSION

Although uncommon in our region, *L. monocytogenes* infection should always be considered in the endocarditis differential diagnosis due to its high pathogenicity and broad environmental distribution, which may increase the frequency of contamination by this organism.

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## REFERENCES

1. Vázquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Domínguez-Bernal G, Goebel W et al. Listeria pathogenesis and molecular virulence determinants. Clin Microbiol Rev [Internet]. 2001 [cited 2013 Jan 10]; 14(3):584-640. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC88991> or <http://cmr.asm.org/content/14/3/584>. long. Review. PubMed PMID: 11432815; PubMed Central PMCID: PMC88991.
2. Allenberger F, Wagner M. Listeriosis: a resurgent foodborne infection. Clin Microbiol Infect. 2009; 16(1):16-23. doi: <http://dx.doi.org/10.1111/j.1469-0691.2009.03109.x>/abstract. Review. PubMed PMID: 20002687.
3. Dias V, Cabral S, Anjo D, Vieira M, Antunes N, Carvalheiras G. Successful management of Listeria monocytogenes pericarditis: case report and review of the literature. Acta Cardiol. 2011 Aug; 66(4):537-8. Review. PubMed PMID: 21894816.
4. Fernández Guerrero ML, Rivas P, Rábago R, Núñez A, de Górgolas M, Martinell J. Prosthetic valve endocarditis due to Listeria monocytogenes. Report of two cases and reviews. Int J Infect Dis. 2004 Mar; 8(2):97-102. Review. PubMed PMID: 14732327.
5. Delvallée M, Ettahar N, Loïez C, Decoene C, Courcol R, Wallet F. An unusual case of fatal pericarditis due to Listeria monocytogenes. Jpn J Infect Dis [Internet]. 2012 Jul [2013 Jan 10]; 65(4):312-4. Available from: [https://www.jstage.jst.go.jp/article/yoken/65/4/65\\_312/\\_article](https://www.jstage.jst.go.jp/article/yoken/65/4/65_312/_article). doi: <http://dx.doi.org/10.7883/yoken.65.312>. Joi: DN/JST. JSTAGE/yoken/65.312. PubMed PMID: 22814153.
6. Summa C, Walker SA. Endocarditis due to Listeria monocytogenes in an academic teaching hospital: case report. Can J Hosp Pharm [Internet]. 2010 Jul [cited 2013 Jan 10]; 63(4):312-4. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2932496>. PubMed PMID: 22478994; PubMed Central PMCID: PMC2932496.



7. Pocar M, Passolunghi D, Moneta A, Donatelli F. Fulminant prosthetic valve endocarditis caused by *Listeria monocytogenes*. *Eur J Cardiothorac Surg* [Internet]. 2009 Dec [cited 2013 Jan 10]; 36(6):1077. Available from: <http://ejcts.oxfordjournals.org/content/36/6/1077>. long. doi: <http://dx.doi.org/10.1016/j.ejcts.2009.06.036>. Epub 2009 Jul 29. PubMed PMID: 19643625.
8. Pierce D, Calkins BC, Thornton K. Infectious endocarditis: diagnosis and treatment. *Am Fam Physician* [Internet]. 2012 May 15 [cited 2013 Jan 10]; 85(10):981-6. Available from: <http://www.aafp.org/afp/2012/0515/p981.html>. Review. PubMed PMID: 22612050.
9. Makaryus A, Yang R, Cohen R, Rosman D, Mangion J, Kort S. A rare case of *Listeria monocytogenes* presenting as prosthetic valve bacterial endocarditis and aortic root abscess. *Echocardiography*. 2004 Jul; 21(5):423-7. doi: <http://dx.doi.org/10.1111/j.0742-8222.2004.03093.x>. PubMed PMID: 15209721.
10. Rocourt J, Schrettenbrunner A, Seeliger HPR. Différenciation biochimique des groupes génomiques des *Listeria monocytogenes* (sensu lato). *Ann Microbiol (Inst Pasteur)* 1983 Jan-Feb; 134A(1):65-71. PubMed PMID: 6405670.
11. Clinical and Laboratory Standards Institute – CLSI (US). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. Approved Standard. 3rd ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2008. M31-A3.28.
12. Soussy CJ, Kuzel R, Courvalin P. Comité de l'Antibiogramme de la Société Française de Microbiologie. Definition and Determination of in vitro Antibiotic Susceptibility Breakpoints for Bacteria in France. *Eur J Clin Microbiol Infect Dis*. 1994 Mar; 13(3):238-46. PubMed PMID: 8050437.
13. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. Phenol/SDS method for plant RNA preparation. In: Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K, editors. *Current Protocol in Molecular Biology*. New York: Wiley; 1987. Unit 4.3.1-4.3.4.
14. Hudson JA, Lake RJ, Savill MG, Scholes P, McCormick RE. Rapid detection of *Listeria monocytogenes* in ham samples using immunomagnetic separation followed by polymerase chain reaction. *J Appl Microbiol*. 2001 Apr; 90(4):614-21. doi: <http://dx.doi.org/10.1046/j.1365-2672.2001.01287.x>. PubMed PMID: 11309074.
15. Chen Y, Knabel SJ. Multiplex PCR for simultaneous detection of bacteria of the genus *Listeria*, *Listeria monocytogenes*, and major serotypes and epidemic clones of *L. monocytogenes*. *Appl Environ Microbiol* [Internet]. 2007 Oct [cited 2013 Jan 10]; 73(19):6299-304. Available from: <http://aem.asm.org/content/73/19/6299>. long or <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2075000>. doi: <http://dx.doi.org/10.1128/AEM.00961-07>. PubMed PMID: 17693562; PubMed Central PMCID: PMC2075000.
16. Borucki MK, Call DR. *Listeria monocytogenes* serotype identification by PCR. *J Clin Microbiol* [Internet]. 2003 Dec [cited 2013 Jan 10]; 41(12):5537-40. Available from: <http://jcm.asm.org/content/41/12/5537>. long or <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC309009>. doi: <http://dx.doi.org/10.1128/JCM.41.12.5537-5540.2003>. PubMed PMID: 14662936; PubMed Central PMCID: PMC309009.
17. Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J Clin Microbiol* [Internet]. 2004 Aug [cited 2013 Jan 10]; 42(8):3819-22. Available from: <http://jcm.asm.org/content/42/8/3819>. long or <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC497638>. PubMed PMID: 15297538; PubMed Central PMCID: PMC497638.
18. Ingianni A, Floris M, Palomba P, Madeddu MA, Quartuccio M, Pompei R. Rapid detection of *Listeria monocytogenes* in foods, by a combination of PCR and DNA probe. *Mol Cell Probes*. 2001 Oct; 15(5):275-80. PubMed PMID: 11735299.
19. Johnson J, Jinneman K, Stelma G, Smith BG, Lye D, Messer J et al. Natural atypical *Listeria innocua* strains with *Listeria monocytogenes* pathogenicity island 1 genes. *Appl Environ Microbiol* [Internet]. 2004 Jul [cited 2013 Jan 10]; 70(7):4256-66. Available from: <http://aem.asm.org/content/70/7/4256>. long or <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC444784>. PubMed PMID: 15240309; PubMed Central PMCID: PMC444784.
20. Liu D, Lawrence ML, Austin FW, Ainsworth AJ. A multiplex PCR for species- and virulence-specific determination of *Listeria monocytogenes*. *J Microbiol Methods*. 2007 Nov; 71(2):133-40. Epub 2007 Aug 28. PubMed PMID: 17884210.
21. Kaur S, Malik SV, Vaidya VM, Barbudde SB. *Listeria monocytogenes* in spontaneous abortions in humans and its detection by multiplex PCR. *J Appl Microbiol*. 2007 Nov; 103(5):1889-96. PubMed PMID: 17953598.
22. Wiedmann M, Bruce JL, Keating C, Johnson AE, McDonough PL, Batt CA. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. *Infect Immun* [Internet]. 1997 Jul [cited 2013 Jan 10]; 65(7):2707-16. Available from: <http://iai.asm.org/content/65/7/2707>. long or <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC175382>. PubMed PMID: 9199440; PubMed Central PMCID: PMC175382.
23. Tamura K, Dudley J, Nei M, Kumar S. MEGA 4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* [Internet]. 2007 Aug [cited 2013 Jan 10]; 24(8):1596-9. Available from: <http://mbe.oxfordjournals.org/content/24/8/1596>. long. doi: <http://dx.doi.org/10.1093/molbev/msm092>. Epub 2007 May 7. PubMed PMID: 17488738.
24. Chun J, Huq A, Colwell RR. Analysis of 16S-23S rRNA intergenic spacer regions of *Vibrio cholerae* and *Vibrio mimicus*. *Appl Environ Microbiol* [Internet]. 1999 May [cited 2013 Jan 10]; 65(5):2202-08. Available from: <http://aem.asm.org/content/65/5/2202>. long or <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC91317>. PubMed PMID: 10224020; PubMed Central PMCID: PMC91317.
25. Cruz CD, Martinez MB, Destro MT. *Listeria monocytogenes*: an infectious agent scarcely known in Brazil. *Alim Nutr*. 2008 Apr-Jun; 19(2):195-206.
26. Charpentier E, Courvalin P. Antibiotic resistance in *Listeria* spp. *Antimicrob Agents Chemother* [Internet]. 1999 [cited 2013 Jan 10]; 43(9):2103-08. Available from: <http://aac.asm.org/content/43/9/2103>. long or <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC89430>. Review. PubMed PMID: 10471548; PubMed Central PMCID: PMC89430.
27. Poroś-Gluchowska J, Markiewicz Z. Antimicrobial resistance of *Listeria monocytogenes*. *Acta Microbiol Pol*. 2003; 52(2):113-29. Review. PubMed PMID: 14594399.
28. Aureli F, Ferrini AM, Mannoni V, Hodzic S, Wedell-Weergaard C, Oliva B. Susceptibility of *Listeria monocytogenes* isolated from food in Italy to antibiotics. *Int J Food Microbiol*. 2003 Jun 25; 83(3):325-30. PubMed PMID: 12745237.
29. Chakraborty T, Ebel F, Wehland J, Dufrenne J, Notermans S. Naturally occurring virulence-attenuated isolates of *Listeria monocytogenes* capable of inducing long term protection against infection by virulent strains of homologous and heterologous serotypes. *FEMS Immunol Med Microbiol*. 1994 Nov; 10(1):1-9. PubMed PMID: 7874073.

30. Geese M, Loureiro JJ, Bear JE, Wehland J, Gertler FB, Sechi AS. Contribution of Ena/VASP proteins to intracellular motility of *Listeria* requires phosphorylation and proline-rich core but not F-actin binding or multimerization. *Mol Biol Cell* [Internet]. 2002 Jul [cited 2013 Jan 10]; 13(7):2383-96. Available from: <http://www.molbiolcell.org/content/13/7/2383.long> or <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC117321/>. doi: <http://dx.doi.org/10.1091/mbc.E02-01-0058>. PubMed PMID: 12134077; PubMed Central PMCID: PMC117321.
31. Chen J, Luo X, Jiang L, Jin P, Wei W, Liu D, Fang W. Molecular characteristics and virulence potential of *Listeria monocytogenes* isolates from Chinese food systems. *Food Microbiol*. 2009 Feb; 26(1):103-11. doi: <http://dx.doi.org/10.1016/j.fm.2008.08.003>. Epub 2008 Oct 18. PubMed PMID: 19028313.
32. Antolín J, Gutierrez A, Segoviano R, López R, Ciguenza R. Endocarditis due to *Listeria*: Description of two cases and review of the literature. *Eur J Intern Med*. 2008 Jun; 19(4):295-6. doi: <http://dx.doi.org/10.1016/j.ejim.2007.06.020>. Epub 2008 Jan 30. PubMed PMID: 18471682.

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