Bactericidal Antibiotics Do Not Appear To Cause Oxidative Stress in *Listeria monocytogenes*

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Oxidative stress can be an important contributor to the lethal effect of bactericidal antibiotics in some bacteria, such as *Escherichia coli* and *Staphylococcus aureus*. Thus, despite the different target-specific actions of bactericidal antibiotics, they have a common mechanism leading to bacterial self-destruction by internal production of hydroxyl radicals. The purpose of the present study was to determine if a similar mechanism is involved in antibiotic killing of the infectious human pathogen, *Listeria monocytogenes*. We treated wild-type *L. monocytogenes* and oxidative stress mutants (Δsod and Δfri) with three different bactericidal antibiotics and found no difference in killing kinetics. In contrast, wild-type *E. coli* and an oxidative stress mutant ($\Delta sodA \Delta sodB$) differed significantly in their sensitivity to bactericidal antibiotics. We conclude that bactericidal antibiotics did not appear to cause oxidative stress in *L. monocytogenes* and propose that this is caused by its noncyclic tricarboxylic acid (TCA) pathway. Hence, in this noncyclic metabolism, there is a decoupling between the antibiotic-mediated cellular requirement for NADH and the induction of TCA enzyme activity, which is believed to mediate the oxidative stress reaction.

Listeria monocytogenes is a food-borne human pathogen that can cause the serious infection listeriosis. The disease primarily affects people with compromised immune systems (elderly adults, neonates, etc.) (30). While the number of cases is low (0.1 to 11.3 per million capita), the fatality rate is very high (20 to 30%) (24). Although *Listeria* species are naturally susceptible to a wide range of antibiotics *in vitro* (28), there is only a limited number of antibiotics that are effective *in vivo*, especially in immunocompromised hosts (14). Hence, patients without underlying diseases have good chances for surviving listeriosis, whereas the disease is difficult to treat in those with underlying diseases (13). *L. monocytogenes* is susceptible to most antibiotics, and the occurrence of acquired resistance is low. Exceptions are nalidixic acid, fosfomycin, and third-generation cephalosporins to which most strains exhibit intrinsic resistance (1, 5, 12). It seems that the tolerance to antibiotics and the frequency of acquired resistance genes may slowly be increasing as a result of selective pressure and horizontal gene transfer, respectively (5, 21). This situation should be followed closely, as such development may have greater consequences for future clinical treatment regimens.

Antibiotics are either bactericidal or bacteriostatic. In *L. monocytogenes*, only a few antibiotics are bactericidal, including aminoglycosides such as gentamicin and sulfonamides such as sulfamethoxazole in combination with trimethoprim (so-called cotrimoxazole) (14). Listeriosis is typically treated with a two-drug combination of a β -lactam (e.g., ampicillin) and an aminoglycoside (e.g., gentamicin) (14, 20). In cases of intolerance to β -lactams, cotrimoxazole or vancomycin/teicoplanin is used (12, 24). The repertoire of listericidal antibiotics is thus limited, and most antibiotics, including β -lactams, that are normally bactericidal merely inhibit growth *in vivo* (13).

Antibiotics have three main targets: DNA replication or repair, protein synthesis, or cell wall turnover. However, in recent years, it has been shown that exposure to bactericidal antibiotics also mediates killing of several bacteria via a more general pathway in which reactive oxygen species (ROS) are generated (7, 11, 19, 31). Thus, Kohanski and coworkers showed that bactericidal antibiot-

ics induced production of hydroxyl radicals, which contributed to the killing of *Escherichia coli* and *Staphylococcus aureus* (18). To reestablish the balance after the primary antibiotic attack on cellular components, the expression of tricarboxylic acid (TCA) cycle genes is upregulated in *E. coli* (18, 19). The bactericidal antibiotics lead to a surge in NADH consumption that then induces a burst in superoxide production (18). Bacteria that are resistant to or develop tolerance to oxidative stress would therefore likely be less sensitive to bactericidal antibiotics. Since *L. monocytogenes* is an intracellular pathogen that repeatedly must survive cellular oxidative burst, in the present study, we questioned whether one reason for the limited bactericidal antibiotic effect could be an inherent resistance to the antibiotic-mediated oxidative stress.

In many bacteria, enzymatic defense systems, including superoxide dismutases (SODs) and catalases/peroxidases, counteract oxidative injuries. In *E. coli*, three different SODs exist, encoded by *sodA* (Mn-SOD), *sodB* (Fe-SOD), and *sodC* (Cu-Zn-SOD), of which the first two SODs are cytosolic enzymes and the third SOD is a periplasmic enzyme (11). In contrast, only a single SOD gene has been identified in *L. monocytogenes*, which encodes a functional manganese SOD (29). SODs facilitate the conversion of superoxide into hydrogen peroxide and hence decrease the level of superoxide but increase the level of hydrogen peroxide (15). Hydrogen peroxide can either be detoxified by the action of catalases/peroxidases or react with ferrous iron (Fe^{2+}) to produce hydroxyl radicals through the Fenton reaction (16). The cascade of ROS reactions causes the generation of hydroxyl radicals, which immediately react with and damage biological components, including nucleic acids, lipids, and amino acids.

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The purpose of the present study was to determine if antibiotics, in addition to the known specific targets, also exert antibacterial effect via oxidative stress in *L. monocytogenes*. If this is not the case, it could be one reason for the limited number of listericidal antibiotics. One way of addressing this is by the use of mutants impaired in oxidative stress response. For instance, *E. coli* mutants impaired in catalytic steps of the oxidative stress response pathway are affected in their susceptibility to antibiotics (7, 11, 31). We therefore hypothesized that *L. monocytogenes* mutants impaired in oxidative stress response mechanisms would also show a differential antibiotic susceptibility compared to their wild-type background if oxidative stress contributed to the antibiotic effect.

MATERIALS AND METHODS

Bacterial strains. *E. coli* strain MG 1655 (3) and the mutant derivative OX 326A (Δ sodA Δ sodB) were included as a positive-control pair, as previous studies have shown oxidative stress in strain MG 1655 upon antibiotic exposure (18). Strain OX 326A lacks cytosolic superoxide dismutase activity due to a deletion of *sodA* (Mn-SOD) and *sodB* (Fe-SOD) (23) and was a kind gift from H. M. Steinman. Experiments with *L. monocytogenes* were performed with strain EGDc (BUG1600) and two oxidative stress response mutants, *L. monocytogenes* EGDc Δ sod (BUG2225) (2) and *L. monocytogenes* EGDc Δ fri (BUG1962) (6), kindly provided by O. Dussurget. Both mutants are deletion mutants, lacking SOD activity and the iron storage protein ferritin, respectively.

Growth conditions and cell enumeration. *L. monocytogenes* strains were grown in brain heart infusion (BHI; Oxoid CM1032), and *E. coli* strains were grown in Luria-Bertani (LB; Difco 244620) under aerobic conditions at 37°C with aeration at 300 rpm. All experiments were performed in light-insulated flasks. Bacterial cell density was determined by spotting 10 μ l in triplicate of a 10-fold dilution series prepared in physiological saline with peptone. Colonies were counted after overnight incubation at 37°C for *L. monocytogenes* and at 25°C for *E. coli*.

Antibiotic killing kinetics. *E. coli* and *L. monocytogenes* were grown overnight in LB and BHI broth, respectively, and a 100-fold dilution made in fresh medium. Growth was continued until an optical density at 600 nm (OD₆₀₀) of approximately 0.1. Five milliliters of bacterial culture was transferred to new flasks, and antibiotics were added at $T = 0$. Oxidative stress controls were performed with the addition of hydrogen peroxide (H₂O₂; Merck catalog no. 1.07209.1000) in concentrations of 5 mM for *E. coli* and 15, 17.5, or 20 mM for *L. monocytogenes*. Survival was determined by bacterial counts at 1-h time intervals ($T = 0, 1, 2$, and 3 h) for the following concentrations of the antibiotics: norfloxacin 250 ng ml⁻¹, gentamicin 10 μ g ml⁻¹, and ampicillin 5 μ g ml⁻¹ for *E. coli* and norfloxacin 20 μ g ml⁻¹, gentamicin 2 μ g ml⁻¹, and ampicillin 10 μ g ml⁻¹ for *L. monocytogenes*. The antibiotic concentrations were chosen based on a series of preliminary experiments aiming at a 2- to 4-log kill during the course of the experiment. For *L. monocytogenes*, additional dose-response patterns were measured by determining survival against norfloxacin at concentrations of 5, 10, 40, and 100 μ g ml⁻¹ for 4 h, gentamicin at 3, 5, 10, and 15 μ g ml⁻¹ for 2 h, and kanamycin at 10, 15, 20, 25, and 40 μ g ml⁻¹ for 2 h. The killing kinetics of all bactericidal antibiotics was tested in duplicate experiments as a minimum. Killing kinetics of ampicillin in *L. monocytogenes* was performed only once, as ampicillin is bacteriostatic to *L. monocytogenes*, but higher concentrations (20 and 30 μ g ml⁻¹) were also tested.

Statistics. Differences among strain susceptibility to hydrogen peroxide or antibiotics were calculated using the Student *t* test on log-transformed percent survival. Experiments with wild-type and mutant strains were run simultaneously, and the survival of strains tested in simultaneous experiments was used as a pairwise measure and compared in a one-tailed paired *t* test with *n* representing the number of replicate experiments.

FIG 1 Survival of exponentially growing *E. coli* treated with 5 mM H₂O₂ (A) and *L. monocytogenes* treated with 20 mM H₂O₂ (B). Symbols: wild type treated with H₂O₂ (filled circles) and without H₂O₂ (open squares), Δ sod mutant treated with H₂O₂ (open circles) and without H₂O₂ (filled diamonds), and Δ fri mutant treated with H₂O₂ (open triangles) and untreated control (filled triangles). Three replicates were performed for each experiment, and representative data are shown.

RESULTS

Comparative studies of antibiotic killing kinetics were performed to analyze the importance of key oxidative stress response genes for the susceptibility of *L. monocytogenes* and *E. coli* to bactericidal antibiotics. The wild-type strains *L. monocytogenes* EGDc and *E. coli* MG 1655 were compared with mutant derivatives deficient in SOD activity as well as with *L. monocytogenes* deficient in the ferritin iron storage protein.

Sensitivity of the wild type and oxidative stress mutants to hydrogen peroxide. To establish that the mutants had changed susceptibility profiles toward oxidative stress compared to the wild types, we first treated the bacterial cultures with the oxidative agent hydrogen peroxide. The *E. coli* Δ sodA Δ sodB mutant was killed faster by 5 mM hydrogen peroxide than the parent wild-type strain, with an up-to-2-log difference in CFU reduction after 1 h of exposure (Fig. 1A). However, the difference was most pronounced at shorter times of exposure, and after 3 h, survival of the mutant and the wild type were similar (Fig. 1A).

L. monocytogenes tolerates higher concentrations of hydrogen peroxide than *E. coli*. Fifteen millimolars hydrogen peroxide was bacteriostatic (data not shown); however, survival decreased drastically with concentrations increasing up to 20 mM hydrogen peroxide, where large differences between wild-type and mutant strains were observed (Fig. 1B). The Δ fri mutant was more susceptible to hydrogen peroxide and had a significantly lower survival than the wild type ($P < 0.05$) (Fig. 1B). Also, the Δ sod mutant exhibited a changed oxidative resistance profile; however, this mutant was significantly more tolerant to hydrogen peroxide than the wild type ($P < 0.05$).

Sensitivity of the wild type and oxidative stress mutants to antibiotics. In *E. coli*, the oxidative stress response pathway involving superoxide dismutase activity had a pronounced effect on

FIG 2 Survival of exponentially growing *E. coli* treated with 250 ng ml⁻¹ norfloxacin, 10 µg ml⁻¹ gentamicin, or 5 µg ml⁻¹ ampicillin. Symbols: wild type treated with antibiotics (filled circles) and without antibiotics (open squares) and Δ sodA Δ sodB mutant OX 326A treated with antibiotics (open circles) and without antibiotics (filled diamonds). All experiments were performed in triplicate, and representative data are shown.

antibiotic susceptibility. Treatment of the *E. coli* wild type and the Δ sodA Δ sodB mutant with norfloxacin (250 ng ml⁻¹) or ampicillin (5 µg ml⁻¹) resulted in a significantly larger reduction in the number of CFU in the wild type than in the mutant ($P < 0.01$) (Fig. 2). Hence, after 3 h, the wild type was reduced to approximately 0.01%, whereas the survival of the mutant was approximately 10%. Likewise, the wild type seemed to be more sensitive than the mutant to treatment with gentamicin (10 µg ml⁻¹) (Fig. 2), but there was a greater variation in survival between these experiments and the difference between the strains was not significant.

In contrast, treatment of *L. monocytogenes* with gentamicin, norfloxacin, or ampicillin resulted in very similar survival of the wild type and the Δ sod and Δ fri mutants (Fig. 3). Gentamicin (2 µg ml⁻¹) and norfloxacin (20 µg ml⁻¹) were bactericidal to the *L. monocytogenes* strains, and both antibiotics caused an up-to-2-log reduction in CFU numbers after 3 h. Ampicillin (5 µg ml⁻¹) was bacteriostatic, but for all antibiotics the susceptibility was indiscriminate between wild-type and mutant strains (Fig. 3).

Wang and Zhao (31) reported that the difference in antibiotic

FIG 3 Survival of exponentially growing *L. monocytogenes* treated with 20 µg ml⁻¹ norfloxacin, 2 µg ml⁻¹ gentamicin, or 10 µg ml⁻¹ ampicillin. Symbols: wild type treated with antibiotics (filled circles) and without antibiotics (open squares), Δ sod mutant treated with antibiotics (open circles) and without antibiotics (filled diamonds), and Δ fri mutant treated with antibiotics (open triangles) and untreated control (filled triangles). Experiments with norfloxacin and gentamicin were performed in triplicate, and representative data are shown. The ampicillin experiment was performed only once.

susceptibility between an *E. coli* wild type and a SOD-deficient mutant increased with increasing concentrations of antibiotics. We therefore exposed the *L. monocytogenes* strains to different concentrations of antibiotics for a fixed period of time to investigate whether the survival of the mutants and the wild type would be differentially influenced at higher concentrations. Although the dose-response experiments resulted in greater variation in survival between the wild-type and mutant strains, no significant differences were found after exposure to gentamicin (3, 5, 10, or 15 µg ml⁻¹) for 2 h or norfloxacin (5, 10, 40, or 100 µg ml⁻¹) for 4 h (Fig. 4). Dose-response experiments were not performed with ampicillin due to its bacteriostatic effect in *L. monocytogenes*. However, kanamycin was included (10, 15, 20, 25, or 40 µg ml⁻¹) for 2 h as a second aminoglycoside in addition to gentamicin. Kanamycin was previously used to determine the differential susceptibility between the *E. coli* wild type and oxidative stress mutants (31). In *L. monocytogenes*, no significant susceptibility differences between the wild type and oxidative stress mutants were detected against this drug either (Fig. 4).

FIG 4 Dose-response curves of exponentially growing *L. monocytogenes* treated with different concentrations of norfloxacin for 4 h, gentamicin for 2 h, or kanamycin for 2 h. Cell density at time 0 equals 100%. Symbols: wild type, filled circles; Δ sodA mutant, open circles; and Δ fri mutant, open triangles. Experiments were performed in duplicate as a minimum, and representative data are shown.

DISCUSSION

The contribution of oxidative stress to the lethal effect of bactericidal antibiotics has been documented for a number of species, including *E. coli*, *S. aureus*, and *Enterococcus faecalis* (4, 7, 11, 31). The purpose of the present study was to assess whether antibiotic-mediated oxidative stress also contributes to the killing of the important human pathogen *L. monocytogenes*.

In agreement with earlier studies (7, 31), we found that bactericidal antibiotics in *E. coli* exert a target-nonspecific effect likely involving the oxidative stress response, since mutants impaired in the oxidative stress response and the wild type survived antibiotic treatment differently. However, this was not the case for *L. monocytogenes*, where the antibiotic susceptibilities of *L. monocytogenes* EGDe Δ sod and Δ fri mutants were almost identical.

SOD and the ferritin iron storage protein are central to the *L.*

monocytogenes defense against oxidative stress (2, 6, 9, 22), and, as expected, we found that survival of the mutants was significantly different from that of the wild type when challenged with 20 mM hydrogen peroxide. The Δ fri mutant had a lower survival rate than the wild type, which correlated well with previous studies showing hypersensitivity to hydrogen peroxide and oxidative stress and a significantly lower tolerance to the superoxide-generating agent plumbagin (6, 22). In contrast, the *L. monocytogenes* Δ sod strain was significantly less susceptible to 20 mM hydrogen peroxide than the wild type. This result was unexpected, since the Δ sod strain has been shown to be more susceptible to oxidative stress as evidenced by having a larger inhibition zone when challenged with hydrogen peroxide or paraquat in a disc diffusion assay (2). We speculate that these inconsistent results in susceptibility of the wild type and the Δ sod strain are due to the dual role of SOD in balancing the account of oxidative agents. Hence, disablement of SOD may be a disadvantage at low concentrations of hydrogen peroxide, due to buildup of superoxide in the mutant, while the capacity of SOD and catalases in the wild type is sufficient to catalyze both the conversion of superoxide and hydrogen peroxide. Alternatively, at high concentrations of hydrogen peroxide, the disablement of SOD may be an advantage, due to the suppression of hydrogen peroxide generation and following the containment of the Fenton reaction (31).

When challenged with any of three different antibiotics, norfloxacin (DNA inhibitor), gentamicin (protein inhibitor), or ampicillin (cell wall inhibitor), neither the *L. monocytogenes* Δ fri mutant nor the Δ sod mutant showed a differential susceptibility compared to the wild type. To verify that SOD inactivation could in fact have an influence on the antibiotic susceptibility, the *E. coli* wild-type strain and the Δ sodA Δ sodB mutant were included as positive controls. Corresponding with previous results, the Δ sodA Δ sodB mutant was less susceptible to hydrogen peroxide than the wild type (23). When we compared the susceptibilities of wild-type *E. coli* and the Δ sodA Δ sodB mutant against ampicillin, norfloxacin, or gentamicin, the mutant exhibited significantly higher survival rates. This is in line with a previous study showing a 10- to 100-fold increase in survival of an *E. coli* Δ sodA Δ sodB mutant against norfloxacin, ampicillin, or kanamycin compared to the wild type (31). This demonstrates that lack of SOD activity can protect *E. coli* from antibiotic-mediated oxidative stress. In contrast, the same effect is not apparent in *L. monocytogenes*. Together, these results imply that (i) antibiotic-imposed oxidative reactions in *L. monocytogenes* do not involve the ferritin iron storage protein and SOD, (ii) *L. monocytogenes* is able to compensate for the lack of these proteins in oxidative defense, or (iii) *L. monocytogenes* does not, in our experimental setup, experience antibiotic-imposed oxidative stress.

Bacterial response to bactericidal antibiotics has previously been shown to be tightly coupled to the activity of enzymes present in the tricarboxylic acid (TCA) cycle (18). In *E. coli*, bactericidal antibiotics increase the expression of TCA genes, and it is this increase in metabolic activity which is hypothesized to be the onset of a lethal oxidative stress reaction (19). The TCA cycle supplies the electron transfer chain with reducing equivalents. A by-product of the electron transfer chain is superoxide, which targets intracellular iron-sulfur clusters and releases ferric iron. Ultimately, this fuels the production of deleterious hydroxyl radicals via the Fenton reaction (18). In contrast to *E. coli* and *S. aureus*, which are the organisms most intensively studied with respect to

the antibiotic-mediated oxidative stress theory, *L. monocytogenes* possess a noncyclic TCA pathway due to lack of α -ketoglutarate dehydrogenase (8, 10, 27). The TCA cycle is an important source of ATP, reducing equivalents, and biosynthetic precursors and thus serves both catabolic and anabolic functions. However, in bacteria such as *Listeria*, *Lactococcus*, and *Clostridium* lacking α -ketoglutarate dehydrogenase (17), the metabolic activity occurs, as it does for *E. coli* grown under anaerobic conditions (26), via a split two-way pathway (27). This pathway consists of an oxidative TCA branch (citrate synthase, aconitase, and isocitrate dehydrogenase) producing α -ketoglutarate and a reductive branch producing succinyl-coenzyme A (25).

We suggest that the above-described differences in TCA metabolism may be responsible for the lack of difference in sensitivity of wild-type *L. monocytogenes* and oxidative stress mutant strains to bactericidal antibiotics. Hence, if no or only low levels of oxidative stress are induced in *L. monocytogenes* during antibiotic exposure, no difference in oxidative stress mutants' behavior would be expected. One could hypothesize that this limitation on antibiotic-mediated oxidative stress in bacteria could influence the potency of antibiotics. Further studies of *L. monocytogenes* metabolism and redox state during antibiotic exposure are required to validate this hypothesis. One such study could include *L. monocytogenes* mutants genetically modified by insertion of α -ketoglutarate dehydrogenase to obtain a full TCA cycle.

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REFERENCES

- Aarestrup FM, Knochel S, Hasman H. 2007. Antimicrobial susceptibility of *Listeria monocytogenes* from food products. *Foodborne Pathog. Dis.* 4:216–221.
- Archambaud C, Nahori MA, Pizarro-Cerda J, Cossart P, Dussurget O. 2006. Control of *Listeria* superoxide dismutase by phosphorylation. *J. Biol. Chem.* 281:31812–31822.
- Bachmann B. 1987. Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12, p 1190–1219. In Neidhardt FC, et al. (ed), *Escherichia coli and Salmonella Typhimurium: cellular and molecular biology*. American Society for Microbiology, Washington, DC.
- Bizzini A, Zhao C, Auffray Y, Hartke A. 2009. The *Enterococcus faecalis* superoxide dismutase is essential for its tolerance to vancomycin and penicillin. *J. Antimicrob. Chemother.* 64:1196–1202.
- Conter M, et al. 2009. Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. *Int. J. Food Microbiol.* 128:497–500.
- Dussurget O, et al. 2005. *Listeria monocytogenes* ferritin protects against multiple stresses and is required for virulence. *FEMS Microbiol. Lett.* 250: 253–261.
- Dwyer DJ, Kohanski MA, Hayete B, Collins JJ. 2007. Gyrase inhibitors induce an oxidative damage cellular death pathway in *Escherichia coli*. *Mol. Syst. Biol.* 3:91.
- Eisenreich W, et al. 2006. ^{13}C isotopologue perturbation studies of *Listeria monocytogenes* carbon metabolism and its modulation by the virulence regulator PrfA. *Proc. Natl. Acad. Sci. U. S. A.* 103:2040–2045.
- Fisher CW, et al. 2000. Influence of catalase and superoxide dismutase on ozone inactivation of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 66:1405–1409.
- Glaser P, et al. 2001. Comparative genomics of *Listeria* species. *Science* 294:849–852.
- Goswami M, Mangoli SH, Jawali N. 2006. Involvement of reactive oxygen species in the action of ciprofloxacin against *Escherichia coli*. *Antimicrob. Agents Chemother.* 50:949–954.
- Hof H. 2003. Therapeutic options. *FEMS Immunol. Med. Microbiol.* 35:203–205.
- Hof H, Nichterlein T, Kretschmar M. 1997. Management of listeriosis. *Clin. Microbiol. Rev.* 10:345–357.
- Hof H, Waldenmeier G. 1988. Therapy of experimental listeriosis—an evaluation of different antibiotics. *Infection* 16(Suppl 2):S171–S174.
- Imlay JA. 2003. Pathways of oxidative damage. *Annu. Rev. Microbiol.* 57:395–418.
- Imlay JA. 2008. Cellular defenses against superoxide and hydrogen peroxide. *Annu. Rev. Biochem.* 77:755–776.
- Karlin S, Theriot J, Mrazek J. 2004. Comparative analysis of gene expression among low G+C gram-positive genomes. *Proc. Natl. Acad. Sci. U. S. A.* 101:6182–6187.
- Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. 2007. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* 130:797–810.
- Kohanski MA, Dwyer DJ, Wierzbowski J, Cottarel G, Collins JJ. 2008. Mistranslation of membrane proteins and two-component system activation trigger aminoglycoside-mediated oxidative stress and cell death. *Cell* 135:679–690.
- Lamont RF, et al. 2011. Listeriosis in human pregnancy: a systematic review. *J. Perinat. Med.* 39:227–236.
- Morvan A, et al. 2010. Antimicrobial resistance of *Listeria monocytogenes* strains isolated from humans in France. *Antimicrob. Agents Chemother.* 54:2728–2731.
- Olsen KN, et al. 2005. The Dps-like protein Fri of *Listeria monocytogenes* promotes stress tolerance and intracellular multiplication in macrophage-like cells. *Microbiology* 151:925–933.
- Steinman HM. 1992. Construction of an *Escherichia coli* K-12 strain deleted for manganese and iron superoxide dismutase genes and its use in cloning the iron superoxide dismutase gene of *Legionella pneumophila*. *Mol. Gen. Genet.* 232:427–430.
- Swaminathan B, Gerner-Smidt P. 2007. The epidemiology of human listeriosis. *Microbes Infect.* 9:1236–1243.
- Tanaka N, Hanson RS. 1975. Regulation of the tricarboxylic acid cycle in gram-positive, facultatively anaerobic bacilli. *J. Bacteriol.* 122:215–223.
- Thomas AD, Doelle HW, Westwood AW, Gordon GL. 1972. Effect of oxygen on several enzymes involved in the aerobic and anaerobic utilization of glucose in *Escherichia coli*. *J. Bacteriol.* 112:1099–1105.
- Trivett TL, Meyer EA. 1971. Citrate cycle and related metabolism of *Listeria monocytogenes*. *J. Bacteriol.* 107:770–779.
- Troxler R, von Graevenitz A, Funke G, Wiedemann B, Stock I. 2000. Natural antibiotic susceptibility of *Listeria* species: *L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri* and *L. welshimeri* strains. *Clin. Microbiol. Infect.* 6:525–535.
- Vasconcelos JA, Deneer HG. 1994. Expression of superoxide dismutase in *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 60:2360–2366.
- Vázquez-Boland JA, et al. 2001. *Listeria* pathogenesis and molecular virulence determinants. *Clin. Microbiol. Rev.* 14:584–640.
- Wang X, Zhao X. 2009. Contribution of oxidative damage to antimicrobial lethality. *Antimicrob. Agents Chemother.* 53:1395–1402.