



Antimicrobial sensitivity of *Listeria monocytogenes* isolated from beef

Sensibilidade antimicrobiana de Listeria monocytogenes isolada de carne bovina

Rodolfo Nunes de Almeida¹, Ana Cláudia Chesca², Aparecida Donizetti da Silva³, Erika Cecília Vallim Severino⁴, Otávio Augusto Martins⁵, Fernanda Raghianti^{6*}

Abstract: The objective of this study was to investigate the antimicrobial susceptibility of *Listeria monocytogenes* previously isolated from beef. The experiment used ground beef, commercialized in the city of Uberaba, Minas Gerais, Brazil. Using the Kirby-Bauer method, analyzes of antimicrobial susceptibility were carried out in the 14 isolates of *Listeria monocytogenes*. All strains (100%) showed sensitivity to antimicrobials: amoxicillin, ampicillin, gentamicin, penicillin, teicoplanin and vancomycin; Six strains (42.86%) presented sensitivity to sulfamethoxazole / trimethoprim and tetracycline; 3 strains (21.43%) presented sensitivity to erythromycin and 2 strains (14.29%) showed sensitivity to ciprofloxacin. While 8 strains (57.14%) presented intermediate resistance to ciprofloxacin and tetracycline. All strains isolated showed resistance to cefazolin, cefepime, cefotaxime, clindamycin, oxacillin and polymyxin b. The antimicrobial resistance of *Listeria monocytogenes* poses serious risks to public health, due to its seriousness in affecting vulnerable segments of the human population, due to the wide presence of this bacterium in the environment, being isolated from food processing environments, water effluents to industries and causing possible contamination of various foodstuffs.

Index terms: Listeriosis, food safety, public health.

Resumo: O objetivo deste estudo foi investigar a sensibilidade antimicrobiana de *Listeria monocytogenes* previamente isolada de carne bovina. O corte de carne bovina usada no experimento foi acém, moído, comercializado no município de Uberaba, Minas Gerais, Brasil. Utilizando o método de Kirby-Bauer, foram realizadas nas 14 cepas isoladas de *Listeria monocytogenes* as análises de suscetibilidade aos antimicrobianos. Todas as cepas (100%) apresentaram sensibilidade aos antimicrobianos: amoxicilina, ampicilina, gentamicina, penicilina, teicoplanina e vancomicina; 6 cepas (42,86%) apresentaram sensibilidade ao sulfametoxazol/trimetoprim e tetraciclina; 3 cepas (21,43%) apresentaram sensibilidade a eritromicina e 2 cepas (14,29 %) apresentaram sensibilidade a ciprofloxacina. Enquanto que 8 cepas (57,14%) apresentaram resistência intermediária a ciprofloxacina e tetraciclina. Todas as cepas isoladas apresentaram resistência a cefazolina, cefepime, cefotaxima, clindamicina, oxacilina e polimixina b. A resistência antimicrobiana de *Listeria monocytogenes* ocasiona sérios riscos à saúde pública, por sua gravidade em acometer segmentos vulneráveis da população humana, pela ampla presença desta bactéria no ambiente, sendo isolada de ambientes de processamento alimentar, efluentes de água para indústrias e por ocasionar eventual contaminação de diversos gêneros alimentícios.

Termos para indexação: Listeriose, segurança alimentar, saúde pública.

Corresponding author: E. Mail: * fernanda.raghianti@iftm.edu.br

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¹Especialista, Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro (IFTM), Campus Uberlândia, Minas Gerais, Brasil. E-mail: rodolfonunes@edu.uniube.br

²Doutorado, Universidade de Uberaba (UNIUBE), Uberaba, Minas Gerais, Brasil. E-mail: ana.chesca@uniube.br

³Graduação, Universidade de Uberaba (UNIUBE), Uberaba, Minas Gerais, Brasil. E-mail:

aparecida.microbiologia@uniube.br

⁴Graduação, Universidade de Uberaba (UNIUBE), Uberaba, Minas Gerais, Brasil. E-mail: erika_vallim@hotmail.com

⁵Pós-Doutorado, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista (UNESP), *Campus* de Botucatu, São Paulo, Brasil. E-mail: oamartins@fmvz.unesp.br

⁶Doutorado, Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro (IFTM), *Campus* Uberlândia, Minas Gerais, Brasil. *E-mail: fernanda.raghianti@iftm.edu.br

Introduction

High humidity, near-neutral pH, nutrient-rich composition, increased surface area and high handling rate during processing give the milled beef an excellent bacterial growth potential by favoring the installation, survival and the multiplication of pathogenic microorganisms, such as *Listeria monocytogenes*, capable of reproducing under refrigeration temperature due to its psychrotrophic characteristics (Jay, 2005; Mantilla et al., 2007; Forsythe, 2013). For this reason, this bacterium can be found frequently in many food products and processing plants as a constituent of biofilms (Filgueiras & Vanetti, 2006, FDA, 2017).

Food-borne listeriosis is a relatively rare and serious disease with a high mortality rate of 20% to 30% produced by opportunistic infection with *Listeria monocytogenes*, affecting specific segments of the population that have some type of impairment in the immune system (FAO WHO, 2004, FDA, 2017). Symptoms such as febrile gastroenteritis in healthy adults after ingestion of large numbers of *L. monocytogenes* cells are common (Bhat et al., 2012).

The incubation period of *L. monocytogenes* varies between 10 and 70 days after oral contamination. The first clinical signs appear unspecific and are constantly confused with flu symptoms. However, gastroenteritis, endocarditis, pneumonia, vomiting, arthritis, ocular diseases, hepatitis, cramps and lack of appetite may occur (Lecuit, 2007; Forsythe,

2013). Cases where the infection manifests itself systemically and reaches the host's nervous system, there are cases of meningitis, encephalitis and abscesses, whereas in pregnant women premature birth, fetal malformations, neonatal sepsis, spontaneous abortion and stillbirth may occur (Franco & Landgraf, 2008, Tortora, 2016).

Many bacterial species can develop resistance to antibiotics to which they are exposed due to the use in clinical and veterinary areas. In general, it occurs due to random mutations with consequent positive selection of antimicrobial resistant mutants, mobilization and sharing by resistance gene transfer and dissemination of strains that have developed previous resistance (Tortora, 2016; Forsythe, 2013). However, in recent years there has been an increase in the scientific reports of *L. monocytogenes* strains, although they have a natural resistance to fosfomycins and cephalosporins resistant to antimicrobials (FDA, 2017).

Considering the high associated mortality rate, the emergence and spread of antimicrobial resistance in *L. monocytogenes* can generate significant clinical implications for the treatment of listeriosis (Conter et al., 2009). Given the importance of this microorganism to public health, the objective of this work was to investigate the antimicrobial profile of 14 strains of *Listeria monocytogenes* previously isolated from samples of ground beef sold in the city of Uberaba-MG.

Material and Methods

Samples

Thirty samples of fresh ground beef, randomly collected from meat retailers in the city of Uberaba-MG, were used. Sample temperature data were collected at the time of collection. The samples were transported in an isothermal box containing reusable ice until it was received at the Food Microbiology Laboratory of the University of Uberaba (UNIUBE), Minas Gerais, Brazil.

Listeria monocytogenes research

To investigate *L. monocytogenes*, the direct plating methodology described by Silva et al. (2017). The isolated strains were phenotypically confirmed by BAM / FDA-Bacteriological Analytical Manual / Food and Drug Administration (2001) and stored at -80 ° C in cryogenic tubes containing 2 mL of 50% BHI (Brain Heart Infusion) solution and glycerol, time of antimicrobial susceptibility testing.

Antimicrobial susceptibility

After slow thawing, the previously isolated and identified strains of *L. monocytogenes* were reactivated by culture in TSB-YE broth (Trypticase Soy Broth supplemented with 0.6% Yeast Extract) at 35°C for 24 h, then pealed at 5% sheep blood agar (Mueller Hinton Agar) for 24 hours at 35°C.

According to guidelines of the Clinical and Laboratory Standards Institute (CLSI M45-A3, 2015) and European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017), using the Kirby-Bauer (1996) method on 5% the susceptibility analyzes of *L. monocytogenes* strains to antimicrobials were carried out: amoxicillin 10 mcg, ampicillin 10 µg, cefazolin 30 mcg, cefepime 30 µg, cefotaxime 30 µg, ciprofloxacin 05 µg, clindamycin 02 µg, erythromycin 15 mcg, gentamicin 10 µg, oxacillin

01 µg, penicillin g (benzylpenicillin) 10 µl, polymyxin b 300 µg, sulfamethoxazole / trimethoprim 1.25 / 23.75 µg (sulfazotrim 25 µg), teicoplanin 30 µg, tetracycline 30 µg and vancomycin 30 µg.

For the positive control of the culture medium and the halos, an antimicrobial susceptibility test (antibiogram) was performed on a *Streptococcus pneumoniae* strain ATCC 49619.

After the incubation period at 35°C for 24-48hs, the reading and interpretation of the results in sensitive, intermediate or resistant were performed according to the break points for the measurement of the size of the specific halos for *L. monocytogenes* described by EUCAST (2017) for penicillin g, ampicillin, erythromycin and sulfamethoxazole / trimethoprim, whereas for the other antimicrobials, due to lack of standardization in susceptibility criteria for *L. monocytogenes*, cut-off points were used according to CLSI recommendations M100-S26 (2016) for *Staphylococcus* spp., with the exception of vancomycin, which was used in the 2012 CLSI table (M100-S22).

Data analysis

The data analysis of this work to evaluate the antimicrobial susceptibility of *L. monocytogenes* previously isolated from bovine milled samples used Microsoft Excel and Microsoft Word. It is worth noting that the statistical analysis using the Anova methodology complemented with the Tukey test was not applied in this type of study. Results were expressed as percentages.

Results and discussion

Listeria monocytogenes was isolated in 14 (46.66%) of the total of 30 samples of ground

beef analyzed. The sensitivity profile of these strains to the various antimicrobials evaluated is shown in Table 1.

Regarding intermediate resistance, 57.14% (n = 8) of the strains isolated in this study had intermediate resistance to ciprofloxacin and tetracycline.

Resistance to cephalosporins is reported by EUCAST (2017) as the specific natural resistance of *L. monocytogenes*, while resistance to polymyxin b is reported by the Comité de L'antibiogramme de La Société Française de Microbiologie (2016) as the natural resistance of Gram bacilli and oxacillin as specific for *L. monocytogenes*.

Table 1. Antimicrobial susceptibility and resistance profile of *L. monocytogenes* strains isolated from ground beef.

Antimicrobial	Susceptibility %	Resistance
	(n)	% (n)
Amoxicilin 10 mcg	100 (14)	0 (0)
Ampicilin 10 µg	100 (14)	0 (0)
Cefazolin 30 mcg	0 (0)	100 (14)
Cefepime 30 µg	0 (0)	100 (14)
Cefotaxime 30 µg	0 (0)	100 (14)
Ciprofloxacin 05 µg	14,29 (2)	28,57 (4)
Clindamycin 02 µg	0 (0)	100 (14)
Eritromycin 15 mcg	21,43 (3)	78,57 (11)
Gentamicin 10 µg	100 (14)	0 (0)
Oxacilin 01 µg	0 (0)	100 (14)
Penicilin 10 UI	100 (14)	0 (0)
Polimyxin B 300 µg	0 (0)	100 (14)
Sulfamethoxazole / Trimethoprim 1,25/23,75 µg	42,86 (6)	57,14 (8)
Teicoplanin 30 µg	100 (14)	0 (0)
Tetracycline 30 mcg	42,86 (6)	0 (0)
Vancomycin 30 µg	100 (14)	0 (0)

The results of this work, obtained from the analyzes of the 14 isolates of *L. monocytogenes*, are consistent with similar reports in the published scientific literature, such as Hernández & Hernández-Godoy (2004), analyzing the antimicrobial profile of 30 strains of *L. monocytogenes* isolated in the province of Valencia between 1998 and 2003 from foods of

animal origin (fresh meat, fresh sausage, fresh sausages, hamburgers, meatballs and cooked ham) for human consumption showed sensitivity to all antibiotics (30 µg amikacin, 30 µg amoxicillin-clavulanic, 10 µg ampicillin, 30 µg cephalothin, 5 µg ciprofloxacin, 30 µg chloramphenicol, 15 µg erythromycin, 10 µg streptomycin, 10 µg gentamycin , kanamycin 30

µg, nitrofurantoin 300 µg, penicillin G 10 µg, tetracycline 30 µg, ticarcillin 75 µg, tobramycin 10 µg, sulfamethoxazole / trimethoprim 1.25 ± 23.75 µg and vancomycin 30 µg), with the exception of one strain (3.33%), which was resistant to tetracycline.

Similarity also found by Ennaji et al. (2008) in 10 strains of *L. monocytogenes* isolated from raw ground red meat, meat products (sausages) and raw farmed poultry in Morocco, found sensitivity to amoxicillin, ticarcillin, gentamicin, tobramycin, amikacin, chloramphenicol, penicillin and ampicillin and resistance nalidixic acid, colistin and second and third cephalosporins generation.

However, Mantilla et al. (2008), analyzing the antimicrobial profile in *L. monocytogenes* strains from ground beef samples, found 100% antimicrobial resistance clindamycin, oxacillin, gentamicin, sulfazotrim, cefoxitin and ampicillin, 83.3% erythromycin, 66.7 % to tetracycline, penicillin, cephalothin and ciprofloxacin and 50% resistance to amikacin, vancomycin, chloramphenicol and rifampicin. They also reported that 16.7% of strains presented an intermediate profile to ciprofloxacin, chloramphenicol and rifampicin and sensitivity to erythromycin and ciprofloxacin; sensitivity to tetracycline, penicillin, cephalothin, chloramphenicol and rifampicin in 33.3% and amikacin and vancomycin in 50% of *L. monocytogenes* strains isolated in the study.

Chen et al. (2010) in 86 strains of *L. monocytogenes* isolated from fresh catfish fillets and their processing facilities (processing facilities - aquarium and water, food contact surfaces and instruments, and floor) found

resistance to cefotaxime in 71% (n=61) and clindamycin in 69% (n=59), intermediate profile to cefotaxime in 29% (n=25), clindamycin 31% (n=27) and penicillin 83% (n=71) and streptomycin in 2% (n=2) and penicillin sensitivity in 17% (n=15), streptomycin 98% (n=84) and ampicillin, cephalothin, chlorafenicol, erythromycin, gentamicin, kanamycin, oxytetracycline, rifampicin, tetracycline, sulfamethoxazole / trimethoprim and vancomycin in 100% (n=86).

Khen et al. (2014) investigated *L. monocytogenes* strains isolated from meat chain samples (bovine hides, precooled carcasses and ground beef) in Ireland, where a small portion (<5%) of the isolates showed resistance to antimicrobials including ampicillin, vancomycin and gentamicin, which are the recommended treatment options for listeriosis.

Palma et al. (2016), analyzing the antimicrobial profile of 11 strains of *L. monocytogenes* isolated from bovine meat cuts and 2 strains of cattle slaughterhouse environments in the Federal District, identified 100% of strains isolated from beef cattle cuts (n=11) sensitivity to amoxicillin, cephalosporins, chloramphenicol, penicillin, tetracycline and vancomycin, norfloxacin 81.8% (n=9), erythromycin and gentamicin 90.9% (n=10), 63.6% (n=7) a ciprofloxacin and sulphonamides 27.3% (n=3), reported intermediate profile to ciprofloxacin in 27.3% (n=3) and norfloxacin 18.2% (n=2) in 100% (n=11) showed resistance to nalidixic acid, in 72.7% (n=8) the sulfonamides and in 9.1% (n=1) resistance to ciprofloxacin, erythromycin and gentamicin. In another study, evaluating strains isolated from cattle slaughtering environments (drainage streams), the same

authors identified that 100% of them had sensitivity to amoxicillin, cephalosporins, chloramphenicol, erythromycin, gentamicin, norfloxacin, penicillin, tetracycline and vancomycin and 50% to ciprofloxacin.

Concern about human listeriosis caused by antimicrobial resistant *L. monocytogenes* is due to its ability to infect susceptible (immunocompromised) populations through the diet and may become invasive, causing several complications (Jay, 2005). In this sense, Bhat et al. (2012) investigated the antimicrobial profile of *L. monocytogenes* strains isolated from 60 human samples of blood and cerebrospinal fluid in patients with encephalitis and meningitis and observed sensitivity in 100% of the isolates for gentamicin, doxycycline, ciprofloxacin and ceftriaxone, 50% (100%) and ampicillin /cloxacillin (100%), while all strains isolated were resistant to ampicillin, oxytetracycline, amoxicillin, streptomycin, amikacin,

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cefpodoxime, erythromycin, norfloxacin, and cefalexin. cefotaxime.

Listeria spp. (including *L. monocytogenes*) are widely distributed in nature and may be found in soil, human and animal fecal material, sewage, river water, slaughterhouse effluent, food processing environments, poultry, fish and domestic animals. eventually contaminate food (Tortora, 2016).

Conclusion

Listeria monocytogenes present in food can pose serious public health risks. This fact is aggravated when this microorganism presents resistance to several antimicrobials used in the conventional treatment of people affected by listeriosis. Effective control of the quality of food production, especially of animal origin, is extremely important in the prevention of food infections.

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