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# Antibiotic and Bacteriocin Sensitivity of *Listeria monocytogenes* Strains Isolated from Different Foods

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## ABSTRACT

This study aimed to determine the antibiotic and bacteriocin sensitivity of Listeria monocytogenes strains isolated from animal derived foods. With disc diffusion assay, all fourteen *L. monocytogenes* strains were susceptible to the antibiotics, including penicillin G, vancomycin, tetracycline, chloramphenicol, rifampicin, erythromycin, gentamicin and trime- thoprim. However, the percentages of fosfomycin and streptomycin resistances were 92.9% and 7.1%, respectively. Multiple resistances were not observed among the tested strains. The results of well diffusion assays showed that all strains were inhibited by the cell-free supernatant of a bacteriocin-producing strain, *Pediococcus acidilactici* 13, with the inhibition zones ranging from 16.00 to 24.50 mm. These results provide useful information on antibiotic resistance of *L. monocytogenes* strains isolated from foods, and can potentially be used to develop bacteriocin-based interventions to guard against the hazards associated with *L. monocytogenes* in ready-to-eat meat and poultry products.

**Keywords:** *Listeria*; Antibiotic; Bacteriocin

## 1. Introduction

*Listeria monocytogenes* is one of the most important food-borne pathogens due to its widespread distribution in nature [1,2]. Consumption of food contaminated with this pathogen can lead to listeriosis with high rates of morbidity and mortality (25% - 30% overall) [3-5]. Raw meat, dairy foods, non pasteurized milk and soft or semi-soft cheeses, vegetables and fish are major sources of outbreaks of listeriosis [6,7]. Also, ready-to-eat (RTE) foods [8-10] may act as vehicles of transmission of this pathogen. Therefore, all available technological and hygienic prophylactic measures must be adopted to eliminate any chance of contamination of foods with this pathogen.

Currently, non-thermal preservation methods are of growing interest as alternative treatments, especially high intensity pulsed electric fields (HIPEF), high pressure (HP) [11] and the addition of natural antimicrobial substances such as lactic acid and other end products of LAB metabolism, including hydrogen peroxide, diacetyl, acetoin and other organic acids [12]. One promising method among these prevention methods to control *L. monocytogenes*, is using bacteriocins as antimicrobial agent. Bacteriocin and similar metabolites produced by certain lactic acid bacteria, isolated from different types of foods, have been known to inhibit the growth of *Listeria monocytogenes* [13-15] and these antimicrobial peptides can be considered as alternatives to the use of chemical preservatives [16].

Although human listeriosis is very rare, when it occurs it can be fatal or causes serious health problems especially in the susceptible population groups, including the elderly people, pregnant women, fetuses, neonates and immunocompromised individuals [10]. Ampicillin or ampicillin in combination with an aminoglycoside such as streptomycin or gentamicin is the primary choice for therapy [17]. The antibiotic resistance of the pathogen is a significant public health concern [18]. The first antibiotic resistant *L. monocytogenes* strain was reported in 1988. Since then, an increasing number of resistant strains isolated from foods, animals and humans have been reported [19-21]. The studies in the last decade have provided sufficient evidence to document that *Listeria* spp. including *L. monocytogenes* are resistant to various anti-

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biotics such as rifampin, cephalothin, nalidixic acid, penicillin G, sulphamethoxole/trimethoprim, chloramphenicol, tetracycline, oxacillin, lincomycin, flumequine, clindamycin, cefotaxime, cephalosporine, ampicillin, erythromycin, gentamycin, methicillin, teicoplanin, tetracycline and vancomycin [4,5,7,21-25]. Such studies are of significant public health significance since it is of concern that there is a worldwide increase in antibiotic resistance of *L. monocytogenes*. It is suggested that the increased use of antibiotics for therapeutic purposes in animals and humans may lead to the development of antibiotic resistance [22, 26,27]. Since another factor effective on the level of resistance is the geographical differences, it is found necessary to screen the antibiotic resistance patterns of *L. monocytogenes* in food and environmental sources from different geographic areas [27].

In one of our previous studies [13], the bacteriocin producer strain was identified as *Pediococcus acidilactici* by API 50 CHL and 16S rRNA gene sequencing. The bacteriocin activity of the inhibitor substance produced by this strain was 9244 AU/ml. Optimization studies on growth conditions and growth media increased the bacteriocin activity to 204,800 AU/ml. In a study [15], when *Pediococcus* strain was used as a starter culture in sucuk production, the metabolite(s) of the *Pediococcus* strain exhibited antilisterial activity, reduced levels of *L. monocytogenes* and controlled its growth when inoculated post-processing. Therefore, the aim of this study was to evaluate the susceptibility of fourteen *L. monocytogenes* strains isolated from animal-derived food products to a variety of antibiotics and bacteriocin produced by *Pediococcus acidilactici* 13.

## 2. Materials and Methods

### 2.1. Materials

Fourteen *Listeria monocytogenes* strains isolated from raw or cooked foods including sausage, raw minced beef, chicken breast, chicken gizzard, raw meat, raw milk, cooked turkey döner, anchovy and mussel were used in antibiotic and bacteriocin sensitivity tests. These food samples were analyzed according to method of ISO 11290-1 [28] in a previous study [29] and colonies grown on PALCAM agar were collected and identified. Additionally, *L. monocytogenes* ATCC 7644, *L. monocytogenes* RSKK 02028 (Refik Saydam National Public Health Agency, National Type Culture Collection, Ankara, Turkey), *L. monocytogenes* 1505-Viyana and *L. monocytogenes* 1483 (Culture Collection of Food Engineering Department, Hacettepe University, Ankara, Turkey) were used as reference strains. Strain *Pediococcus acidilactici* 13 [13,30] was used to assess bacteriocin sensitivities to the *L. monocytogenes* strains.

### 2.2. Growth of Bacterial Cultures

Bacteriocin-producing strain *P. acidilactici* 13 was propagated in Tryptone Glucose Yeast Extract Broth (TGE; prepared from formulation according to DIFCO Tryptone Glucose Extract Medium) at 37˚C for 24 hours. Stock cultures were maintained at –20˚C in TGE broth containing 15% (v/v) glycerol. Pathogenic strains, *Listeria monocytogenes*, were activated in Tryptic Soy Broth (TSB, MERCK, Darmstadt, Germany) at 30˚C for 24 h.

### 2.3. Antibiotic Disc Diffusion Susceptibility Test

Disk diffusion susceptibility tests were performed according to Clinical and Laboratory Standards Institute standard reference procedure [31]. Three to five well isolated colonies of *L. monocytogenes* were transferred into 10 ml Tryptic Soy Broth (TSB, Merck), incubated at 37˚C for 24 hours, diluted 1:10 in 9 ml 0.1% peptone water (Merck) to a turbidity equivalent to 0.5 McFarland standard (approximately $10^8$ cfu/ml), and inoculated onto the entire surface of a dried Mueller-Hinton Agar (MHA, Merck) plate using a sterile cotton swab. The MHA plates were held at room temperature under a biological hood for 10 minutes to allow evaporation/adsorption of free surface liquid.

Antibiotic discs containing 10 µg streptomycin (S10), 30 µg vancomycin (VA30), 120 µg gentamicin (CN120), 50 µg fosfomycin (FOS50), 5 µg rifampicin (RD5), 30 µg tetracycline (TE30), 10 Units penicillin G (P10), 15 µg erythromycin (E15), 30 µg chloramphenicol (C30), 5 µg trimethoprim (W5) (Oxoid) were placed on the surface of each inoculated MHA plate. After incubation for 24 h at 37˚C, the diameter (in mm) of the zone around each disk was measured and interpreted in accordance with the Clinical and Laboratory Standards Institute Standards guidelines [31], to classify the antibiotic sensitivity of each isolate. *Staphylococcus aureus* ATCC 6538 was used as standard strain.

### 2.4. Well Diffusion Assay

The antimicrobial activity of bacteriocin-producing *P. acidilactici* 13 was tested against 18 *L. monocytogenes* strains by well diffusion assay [32]. For this study, 24 h TGE Broth culture of the bacteriocin-producing isolate was centrifuged at 3214 × g for 10 minutes. Thereafter, the supernatant fluid was collected and filtered through a 0.45 µm pore-size filter (Sartorius, Germany). Wells of 8 mm diameter were cut on TGE Agar plates, and 50 µl portions from the culture supernatant filtrates of the strain were placed into the wells and allowed to diffuse into the agar overnight at 4˚C. Portions (50 µl) of 24 h *L. monocytogenes* cultures were inoculated into 8 ml soft TGE Agar at 45˚C and poured onto the plates. Zones of

 

inhibition were measured after incubation at 30˚C for 18 - 24 h.

## 3. Results and Discussion

Antibiotic sensitivities of fourteen *L. monocytogenes* strains isolated from foods were tested with disc diffusion assay against ten different antibiotics. Disc diffusion assay results showed that all strains including reference strains were susceptible to penicillin G, vancomycin, tetracycline, chloramphenicol, rifampicin, erythromycin, gentamicin and trimethoprim (**Table 1**, **Figure 1**). Although *L. monocytogenes* isolates are susceptible to a wide range of antibiotics, this pathogen has a natural resistance to nalidixic acid, fosfomycin and the third generation cephalosporins [33,34]. Moreover, fosfomycin is included in commercial selective media used for *Listeria* detection because of the fosfomycin resistance of this pathogen [19]. Even though the percentage is very low, fosfomycin resistance of *L. monocytogenes* from meat and fish samples has been reported [35]. Only one *L. monocytogenes* strain isolated from turkey döner kebap (7.1%) displayed sensitivity streptomycin was one of three antibiotics that had comparatively higher frequencies (12.2%) than other antibiotics. The resistance percentage of *L. monocytogenes* isolates against streptomycin was determined as 19.30% by Morobe *et al.* [24].

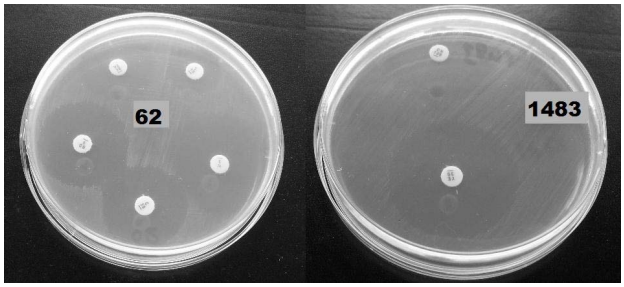


**Figure 1. MHA plates showing the effect of the antibiotics to strain *Listeria monocytogenes* 62 (left) and *Listeria monocytogenes* 1483 (right).**

**Table 1. Antibiotic sensitivity of *Listeria monocytogenes* strains isolated from various foods.**

| Antibiotics | Dose (μg/disc) | Sensitivity of the isolates (%) | | |
|---|---|---|---|---|
| | | R | I | S |
| Penicilin G | 10 IU | 0 | 0 | 100 |
| Vancomycin | 30 | 0 | 0 | 100 |
| Tetracycline | 30 | 0 | 0 | 100 |
| Chloramphenicol | 30 | 0 | 0 | 100 |
| Rifampicin | 5 | 0 | 0 | 100 |
| Erythromycin | 15 | 0 | 0 | 100 |
| Gentamicin | 120 | 0 | 0 | 100 |
| Trimethoprim | 5 | 0 | 0 | 100 |
| Streptomycin | 10 | 7.1 | 0 | 92.9 |
| Fosfomycin | 50 | 92.9 | 0 | 7.1 |

R: resistant, I: intermediate, S: susceptible.

Ampicillin, rifampicin or penicillin alone or in combination with gentamicin is standard antibiotic therapy for listeriosis. Also, trimethoprim with sulfamethoxazole is used especially for patients with allergy to penicillin. The other antibiotics used for the treatment of listeriosis are vancomycin, erythromycin, tetracycline and chloramphenicol [7,14]. In our study, *L. monocytogenes* isolates were highly sensitive to streptomycin (92.9%), vancomycin (100%), gentamicin (100%), rifampicin (100%), tetracycline (100%), penicillin G (100%), erythromycin (100%), chloramphenicol (100%) and trimethoprim (100%) but resistant to fosfomycin (92.9%; **Table 1**). In other words, the *L. monocytogenes* strains tested in the current study were susceptible to the antibiotics used for the treatment of listeriosis.

Another important issue regarding antibiotic resistance of *L. monocytogenes* is their multiple resistances since they may cause failure of antibiotic therapy of listeriosis [7]. Multiple resistant *L. monocytogenes* strains have been reported [27,36]. However, multiple resistances among the isolates tested in the present study were not observed.

*L. monocytogenes* can survive in a wide variety of foodstuffs and its survival times are generally longer at lower temperatures [1]. Application of bacteriocins may help reduce the use of chemical preservatives and/or the intensity of heat and other physical treatments to destroy the pathogens. The resulting minimally processed foods giving home-made appeal to the consumers with least amount of chemical preservatives satisfy the demands of consumers for foods that are fresh tasting, ready to eat, and lightly preserved [37]. Furthermore, pediocin treatment alone or in combination with organic acids showed a stronger effect against survival of *L. monocytogenes* on vegetables [38].

These antimicrobial compounds are mainly used in meat products, but their application to dairy products is being evaluated because of antilisterial activity. These peptides have a wide pH range for activity and they are not affected by heating or freezing [11]. Studies have been focused on the anti-listerial effect of pediocin-like bacteriocins in foods [14,15,39-41]. However, the increased resistance of *Listeria* species against class IIa bacteriocins may limit the potential role of these antimicrobial compounds in biopreservation [42]. Moreover, natural resistance to class IIa bacteriocins has been reported in 1% - 8% of tested wild type strains [43]. We have previously reported [15] resistance of strain 558 of *L. monocyto-*

 

*genes*, a strain isolated from pork [44], to culture supernatants of *P. acidilactici* 13. However, the results in the present study are not in agreement with the study by Cosansu *et al.* [15]. In our study, the results of well diffusion assay showed that all strains tested were inhibited by the cell-free supernatant of a bacteriocin-producing strain, *Pediococcus acidilactici* 13, with the inhibition zones ranging from 16.00 to 24.50 mm (**Table 2**). The largest inhibition zone corresponded to *L. monocytogenes* strain 44 isolated from sausage, while the narrowest inhibition zone was against strain 48 isolated from raw minced beef.

In this study, we report on the antibiotic resistance pattern and bacteriocin sensitivity of 14 *L. monocytogenes* strains. According to antibiotic disk diffusion assay results, all *L. monocytogenes* strains were sensitive to the antibiotics commonly used for treatment of listeriosis. Interestingly, some *L. monocytogenes* strains were resistant to antibiotics: streptomycin and fosfomycin. Regarding sensitivity to the cell free-supernatant of the bacteriocin-producing strain *P. acidilactici* 13, all *L. monocytogenes* strains were found to be inhibited. These results provide useful information on antibiotic resistance of *L. monocytogenes* strains isolated from foods. Further, the findings can potentially be used to develop bacteriocin-based interventions to guard against the hazards associated with *L. monocytogenes* in ready-to-eat meat and poultry products.

**Table 2. The inhibition effect of the bacteriocin produced by *Pediococcus acidilactici* 13 on *L. monocytogenes* strains[*].**

| *Listeria monocytogenes* strains | Inhibition zone diameter (mm) |
|---|---|
| 43 | 22.00 ± 0.00 |
| 44 | 24.50 ± 0.71 |
| 48 | 16.00 ± 5.66 |
| 51 | 19.50 ± 0.71 |
| 56 | 19.50 ± 0.71 |
| 58 | 19.00 ± 2.83 |
| 62 | 19.00 ± 1.41 |
| 78 | 22.50 ± 0.71 |
| 86 | 21.50 ± 2.12 |
| 92 | 23.00 ± 1.41 |
| 99 | 19.00 ± 1.41 |
| 101 | 21.00 ± 1.41 |
| 103 | 22.50 ± 0.71 |
| 107 | 17.00 ± 2.83 |
| ATCC 7644 | 21.50 ± 4.95 |
| 02028 | 21.00 ± 2.83 |
| 1505-Viyana | 21.00 ± 1.41 |
| 1483 | 19.00 ± 2.83 |

[*]Values are means of n = 2 ± SD.

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