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Multidrug resistance cases of *Listeria monocytogenes* isolated from fresh meats†

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Abstract: The aim of this study is to provide an overview of multidrug resistance cases of *Listeria monocytogenes* isolated from fresh meat and meat products, from the North of Portugal. Samples of fresh meat preparations and meat products were undertaken from hypermarkets and small traditional local shops were subjected to microbiological analysis and antimicrobial resistance tests. The strains were identified using morphological and molecular methods. Antibiotic resistance was determined using the Kirby-Bauer disk diffusion method. The overall prevalence of *L. monocytogenes* among screened samples was 32%. A total of 9 isolates obtained from minced meat, displayed a multidrug-resistance profile.

Keywords: *Listeria monocytogenes*; antimicrobial resistance; multidrug resistance; meat

1. Introduction

In the last decades, the selective pressure resulting from the use of antibiotics, has led to the emergence of bacterial strains that exhibit a growing resistance to these agents, with an increase in bacteria showing multi-resistance profiles. *Listeria monocytogenes* is a major bacteria that can contaminate meat and meat products, having an important impact on public health, being a potential cause of foodborne diseases.

The aim of this study is to provide an overview of multidrug resistance cases of *L. monocytogenes* isolated from fresh meat and meat products, from the North of Portugal.

2. Materials and Methods

2.1. Sample Collection and Bacterial Isolates

From april to september 2022, a collection of 75 samples of meat preparations was undertaken, from hypermarkets and small traditional local shops, including fresh meats and meat-based products. Of the total samples, 20% were meat-based products ("alheira" and "moura"), 12% were meatballs and hamburgers, 12% were meat skewers, 8% were breaded meat, 40% were minced meat and 8% were fresh sausage. Samples were transported to the laboratory under refrigeration conditions in 10 minutes, followed by microbiological analysis.

2.2. Sample Collection and Bacterial Isolates

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The antimicrobial susceptibility testing was performed by the Kirby Bauer disk diffusion method, which followed the recommendations given in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2023 guidelines with the exception of kanamycin that followed the Clinical and Laboratory Standards Institute (CLSI) 2017 standards. The following antibiotic discs were used: ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), linezolid (30 μg), meropenem (10 μg), rifampicin (5 μg), tetracycline (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg) and vancomycin (30 μg). A reference strain L. monocytogenes ATCC® was used as a quality control strain.

3. Results

The overall prevalence of *L. monocytogenes* among screened samples was 28%, with highest percentages in meat-based products and meat skewers. The percentage of resistance to each antibiotic is shown in **Figure 1**.

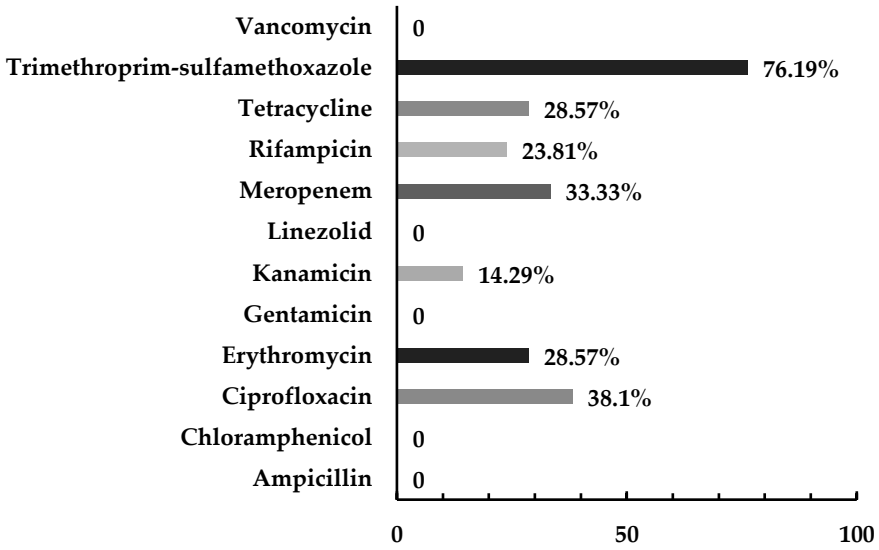

Figure 1. Resistance (%) to each antibiotic by L. monocytogenes isolated from meat and meat products.

There were three strains of *L. monocytogenes* that displayed a multidrug-resistance profile (25%) since they showed resistance to at least three different classes of antimicrobials. Their multidrug-resistance pattern was as follows: one isolate was resistant to four classes of antimicrobials (corresponding to a sample of turkey minced meat) and two isolates were resistant to six classes of antimicrobials (corresponding to a sample of bovine minced meat and turkey minced meat).

Table 1 shows the phenotype of *L. monocytogenes* isolated, regarding their multidrug-antibiotic-resistance.

Table 1. Antimicrobial-resistant phenotype from multidrug-resistant *L. monocytogenes* isolates.

Identification	Multidrug-resistant phenotype
1	RD, CIP, KAN, ERY, MRP, SXT
2	RD, CIP, ERY, TET, MRP, SXT
3	CIP, ERY, MRP, SXT

[1] Abbreviations. RD: rifampicin; CIP: ciprofloxacin, K: kanamycin; ERY: erythromycin; TET: tetracycline, MRP: meropenem; SXT: trimethoprim-sulfamethoxazole.

All three strains have resistance to ciprofloxacin, erythromycin, meropenem and trimethoprim-sulfamethoxazole. Only one strain is resistant to tetracycline and one resistant to kanamycin. Strain number 3 is susceptible to rifampicin.

4. Discussion

In this study, a prevalence of 28% of *L. monocytogenes* in meat and meat products was obtained in retail markets. Research conducted under similar conditions, in meat obtained from retail markets in Brazil and China, show a maximum prevalence of *L. monocytogenes* of 19% (Cavalcanti et al., 2022; Liu et al., 2020).

Regarding antimicrobial resistance, all samples were susceptible to ampicillin, chloramphenicol, gentamicin, linezolid and vancomycin, with no resistance reported. In 2020, Matle et al. published a review about antimicrobial resistance in *L. monocytogenes* from meat and meat products, where they found resistance against most of the antimicrobials that we tested. However, the percentage of resistance is considerably higher in our study, as shown by (Gómez et al., 2014; Maung et al., 2019). Gómez et al. (2014), registered no resistance towards chloramphenicol nor ciprofloxacin and only 0.5% of resistance with tetracycline. Additionally, Maung et al. (2019) encountered no resistance to ampicillin, gentamycin, erythromycin, vancomycin or sulfamethoxazole-trimethoprim and just one case of resistance to linezolid. In our study we found three isolates that showed a multidrug-resistance profile, being resistance to more than three classes of antimicrobials. This percentage is in accordance to a systematic review from Tayeb et al. (2023), that had 22.97% of multidrug-resistance in meat and meat products.

5. Conclusions

A moderate frequency of *L. monocytogenes* (29%) was found among meat and meat products for human consumption. Six strains were multidrug-resistant with a diversity of antimicrobial resistance. These results show us that livestock animals may be an important reservoir of antimicrobial-resistance genes. This is of great concern for public health, since most antimicrobial resistances detected were antimicrobials considered to be essential and frequently used in human medicine. Furthermore, frequent monitoring of strains from livestock is essential to understand the spread and the changes of the genetic repertoire, as well as the zoonotic potential of these strains. Some measures to overcome antimicrobial resistance in meat in Portugal should be taken into consideration, such as the education of livestock producers, limiting the availability of antibiotics and the implementation of more restrictive legislation concerning antimicrobial prescription.

Author Contributions: Conceptualization C.S., V.S. and P.P.; methodology J.P., V.S., P.P. and C.S.; software V.S. and P.P.; validation C.S., V.S. and P.P.; formal analysis J.P., V.S., P.P. and C.S.; investigation J.P., V.S., P.P. and C.S.; resources P.P. and C.S.; data curation J.P., V.S., P.P. and C.S.; writing—original draft preparation J.P. and C.S.; writing—review and editing J.P., V.S., P.P. and C.S.; visualization C.S.; supervision V.S., P.P. and C.S.. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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