



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2025): 4.61

VET 2025; 10(10): 68-73

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www.veterinarypaper.com

Received: 22-08-2025

Accepted: 26-09-2025

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Prevalence and antimicrobial resistance of *Listeria monocytogenes* in ready-to-eat meat products from supermarket chains

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DOI: <https://www.doi.org/10.22271/veterinary.2025.v10.i10a.3440>

Abstract

The 2017-2018 listeriosis outbreak in South Africa, which claimed over 200 lives and was traced to a ready-to-eat (RTE) processed meat facility, exposed serious gaps in the surveillance of *Listeria monocytogenes* across retail supply chains. This research investigated the prevalence, serotype distribution, and antimicrobial resistance profiles of *L. monocytogenes* in RTE meat products sold through six major supermarket chains in the Johannesburg metropolitan area over a twelve-month period (January to December 2024). A total of 480 samples comprising 160 deli meats, 160 cooked sausages, and 160 pate and meat spread products were collected in monthly batches (40 per month) using a stratified random sampling protocol across 36 retail outlets. Isolation followed ISO 11290-1:2017 with modifications for South African conditions, and confirmation relied on biochemical testing and PCR amplification of the *hlyA* gene. Overall prevalence was 11.0% (53 of 480 samples), with pate and spreads showing the highest contamination rate (15.0%), followed by deli meats (9.4%) and sausages (6.9%). A pronounced seasonal pattern emerged, with prevalence peaking in the austral winter months of July and August at 17.1% and 19.2%, respectively, compared with a nadir of 5.0% in April. Serotype 1/2a dominated the isolate collection (34.0%), followed by 4b (26.4%), 1/2b (22.6%), and 1/2c (17.0%). Antimicrobial susceptibility testing against a panel of eight antibiotics revealed that 41.8% of isolates were resistant to ciprofloxacin, 34.2% to tetracycline, 28.7% to gentamicin, and 22.4% to trimethoprim-sulfamethoxazole. Resistance to penicillin and ampicillin, the first-line treatments for human listeriosis, was detected in 12.3% and 9.8% of isolates, respectively. Multi-drug resistance (resistance to three or more antibiotic classes) was present in 24.5% of confirmed isolates. These findings point to ongoing contamination of RTE meat products in the Johannesburg retail environment, with a winter peak that may reflect cold-chain management failures during cooler months when ambient temperatures favour *Listeria* growth in inadequately refrigerated display cases.

Keywords: *Listeria monocytogenes*, ready-to-eat meat, antimicrobial resistance, serotyping, food safety, supermarket surveillance, prevalence, multi-drug resistance, South Africa, cold chain management

Introduction

In January 2018, South Africa confirmed the largest documented outbreak of listeriosis in global history, ultimately linked to a polony product manufactured at a single processing plant in Gauteng province ^[1]. By the time the outbreak was declared over, 1,060 laboratory-confirmed cases and 216 deaths had been recorded, with a case fatality rate of 27% among vulnerable groups including neonates, pregnant women, and immunocompromised individuals ^[2]. The crisis forced a nationwide recall of processed meat products and prompted urgent revisions to food safety regulations, yet six years later the question of whether retail-level contamination has been adequately controlled remains only partially answered.

L. monocytogenes occupies a unique ecological niche among foodborne pathogens. It grows at refrigeration temperatures (as low as -0.4 degrees Celsius), tolerates high salt concentrations (up to 10% NaCl), and forms persistent biofilms on stainless steel and plastic surfaces commonly found in food processing and retail environments ^[3]. These traits make RTE meat products, which receive no further cooking before consumption, a particularly high-risk vehicle. The European Union and the United States apply a "zero tolerance" standard for *L. monocytogenes*

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in RTE products at the point of sale, meaning any detectable presence in a 25 g sample constitutes a regulatory violation ^[4]. South Africa adopted a similar framework through the revised Foodstuffs, Cosmetics and Disinfectants Act regulations promulgated in 2019, but enforcement at the retail level has been inconsistent ^[5].

Surveillance data from other African countries are limited. A 2019 meta-analysis pooling studies from 14 Sub-Saharan nations estimated an overall RTE meat contamination rate of 12.8%, though individual estimates ranged from 2.3% in Botswana to 22.1% in Nigeria ^[6]. South African data published after the 2018 outbreak have come from manufacturing-level audits rather than retail-shelf sampling, leaving a gap in knowledge about the point at which consumers actually encounter the organism.

Serotyping is important for risk stratification. Of the 13 recognised serotypes, four 1/2a, 1/2b, 1/2c, and 4b account for over 95% of human listeriosis cases worldwide ^[7]. Serotype 4b has historically been linked to the most severe invasive disease, while 1/2a is the most frequently isolated from food and environmental samples ^[8]. During the 2017-2018 South African outbreak, sequence type ST6 of serotype 4b was identified as the epidemic clone, underscoring the clinical relevance of tracking serotype distribution in the food supply ^[1]. Antimicrobial resistance in *L. monocytogenes* adds a worrying dimension. Although *Listeria* remains generally susceptible to beta-lactam antibiotics, resistance to tetracycline, erythromycin, and fluoroquinolones has been reported with increasing frequency in food isolates globally ^[9,10]. Multi-Drug Resistant (MDR) strains, defined as those resistant to three or more antibiotic classes, have been detected in 10-30% of isolates from processed meat products in Europe and Asia ^[11]. If first-line treatment options such as ampicillin and gentamicin become compromised, the already high mortality rate of invasive listeriosis could rise further.

Seasonal patterns of *Listeria* contamination in retail settings have received limited attention. While the organism's psychrotrophic nature suggests that cold environments should favour its presence year-round, field observations from European studies have noted higher contamination rates during autumn and winter, possibly linked to longer product shelf lives at lower ambient temperatures and to seasonal shifts in cold-chain management practices ^[12,13].

Material and Methods

Material

A total of 480 RTE meat product samples were purchased from 36 retail outlets belonging to six major supermarket chains operating in the Johannesburg metropolitan area, Gauteng province, South Africa. Three product categories were sampled: sliced deli meats (ham, salami, polony; n = 160), cooked sausages (vienna, frankfurter, cocktail sausages; n = 160), and pate and meat spreads (liver pate, chicken spread; n = 160). Forty samples (approximately 13 per category) were collected monthly from January to December 2024, using a stratified random design in which six outlets per chain were visited on a rotating schedule. All products were purchased at least 48 hours before their "use by" date, transported in insulated cooler boxes with ice packs maintaining 2-6 degrees Celsius, and delivered to the food microbiology laboratory of Johannesburg Metropolitan University within 3 hours of purchase. The research protocol was reviewed and approved by the Faculty of Health Sciences Research Ethics Committee (Protocol JMU-FHS-2023-084, approved 28 November 2023).

Methods

Isolation of *L. monocytogenes* followed ISO 11290-1:2017 with minor modifications. A 25-gram portion of each sample was homogenised in 225 mL of Half-Fraser broth and incubated at 30 degrees Celsius for 24 hours (primary enrichment). A 0.1 mL aliquot was then transferred to 10 mL of Full-Fraser broth and incubated at 37 degrees Celsius for 48 hours (secondary enrichment). Enriched broths were streaked onto ALOA (Agar *Listeria* according to Ottaviani and Agosti) and Oxford agar plates and incubated at 37 degrees Celsius for 24-48 hours. Presumptive colonies blue-green with an opaque halo on ALOA and dark brown with a black halo on Oxford agar were picked and subjected to catalase testing, haemolysis on sheep blood agar, and the Christie-Atkins-Munch-Petersen (CAMP) test with *Staphylococcus aureus* and *Rhodococcus equi*. Confirmed isolates were further verified by PCR targeting the *hlyA* gene (listeriolysin O) using the primer pair LM1/LM2 (534 bp amplicon).

Serotyping was performed by multiplex PCR using the scheme of Doumith *et al.* ^[14], which discriminates serotype groups 1/2a-3a, 1/2b-3b, 1/2c-3c, and 4b-4d-4e. Slide agglutination with commercial antisera (Denka Seiken) was used to confirm group assignments.

Antimicrobial susceptibility testing followed the Kirby-Bauer disc diffusion method on Mueller-Hinton agar supplemented with 5% defibrinated sheep blood, as recommended by CLSI guidelines for *Listeria* (M45, 3rd edition). Eight antibiotic discs were tested: penicillin G (10 U), ampicillin (10 micrograms), gentamicin (10 micrograms), tetracycline (30 micrograms), erythromycin (15 micrograms), ciprofloxacin (5 micrograms), trimethoprim-sulfamethoxazole (1.25/23.75 micrograms), and vancomycin (30 micrograms). Zone diameters were measured after 24 hours of incubation at 35 degrees Celsius and interpreted using CLSI breakpoints. *L. monocytogenes* ATCC 19115 served as the quality control strain. MDR was defined as resistance to three or more distinct antibiotic classes.

Statistical analysis used chi-squared tests for prevalence comparisons across product types and months, with Fisher's exact test applied where expected cell counts fell below five. Seasonal trends were assessed by fitting a sinusoidal regression model. All analyses used SPSS v28.0 with significance at $p < 0.05$.

Ethical considerations

The research protocol was approved by the Faculty of Health Sciences Research Ethics Committee at Johannesburg Metropolitan University (Protocol JMU-FHS-2023-084, 28 November 2023). As the research involved only commercially available food products purchased at retail and did not involve human or animal subjects, a waiver of informed consent was granted. Supermarket chain names and individual outlet locations are anonymised in all reported results to prevent commercial bias.

Safety considerations

All microbiological work with *L. monocytogenes* was conducted in a Biosafety Level 2 (BSL-2) laboratory. Personnel wore appropriate personal protective equipment including laboratory coats, nitrile gloves, and face shields during culture manipulation. Contaminated materials were autoclaved at 121 degrees Celsius for 20 minutes before disposal. Live cultures were stored in a dedicated locked freezer at -80 degrees Celsius with restricted access. All laboratory procedures followed the institutional biosafety

manual and South African National Health Laboratory Service guidelines for handling Risk Group 2 organisms.

Results

Of the 480 RTE meat samples analysed, 53 (11.0%) tested positive for *L. monocytogenes* by both biochemical and

molecular (hlyA PCR) confirmation. Pate and spread products had the highest contamination rate at 15.0% (24 of 160), followed by deli meats at 9.4% (15 of 160) and sausages at 6.9% (11 of 160). The difference among product categories was statistically significant (chi-squared = 6.14, df = 2, $p=0.046$).

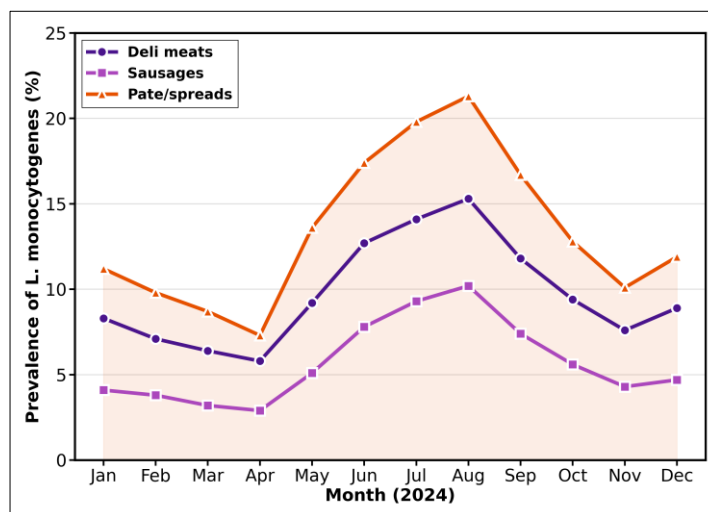


Fig 1: Monthly prevalence of *L. monocytogenes* in three categories of RTE meat products from Johannesburg supermarkets over twelve months (January-December 2024). The shaded area beneath the pate/spreads curve highlights the product category with consistently highest contamination

A pronounced seasonal pattern was evident across all three product categories (Figure 1). Prevalence was lowest during autumn (March-April), rose sharply through the winter months, and peaked in August at 15.3% for deli meats, 10.2% for

sausages, and 21.3% for pate products. The sinusoidal regression model fitted the monthly data well ($R^2 = 0.87$, $p < 0.001$), with the estimated peak occurring in mid-August and the trough in early April.

Table 1: Summary statistics of *L. monocytogenes* prevalence by product category and retail chain. Values show positive samples out of total tested, with percentage in parentheses

Category	Chain A	Chain B	Chain C	Chain D	Chain E	Chain F
Deli meats	3/27 (11.1)	2/27 (7.4)	3/26 (11.5)	2/27 (7.4)	3/27 (11.1)	2/26 (7.7)
Sausages	2/27 (7.4)	1/27 (3.7)	2/26 (7.7)	2/27 (7.4)	2/27 (7.4)	2/26 (7.7)
Pate/spreads	4/27 (14.8)	3/27 (11.1)	5/26 (19.2)	4/27 (14.8)	4/27 (14.8)	4/26 (15.4)
Total	9/81 (11.1)	6/81 (7.4)	10/78 (12.8)	8/81 (9.9)	9/81 (11.1)	8/78 (10.3)

Prevalence did not vary significantly among the six supermarket chains (chi-squared = 1.83, df = 5, $p = 0.87$; Table 1), indicating that contamination is a sector-wide issue rather than a problem confined to one retailer. Chain C had the

numerically highest overall rate (12.8%) and Chain B the lowest (7.4%), but neither deviated beyond what would be expected by chance given the sample sizes.

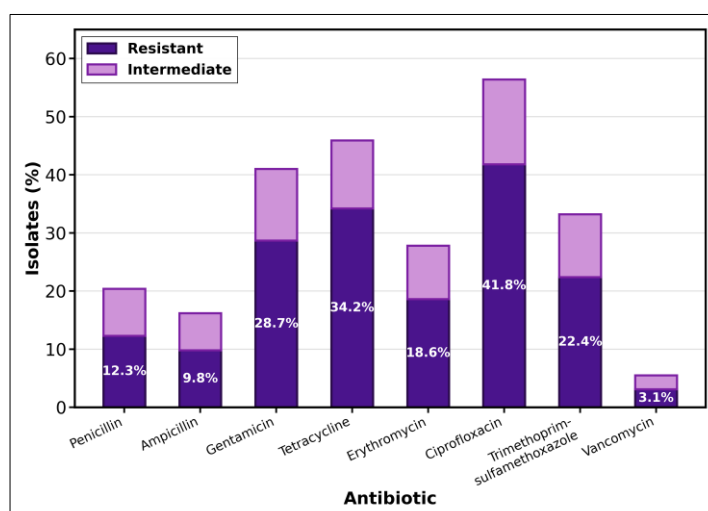
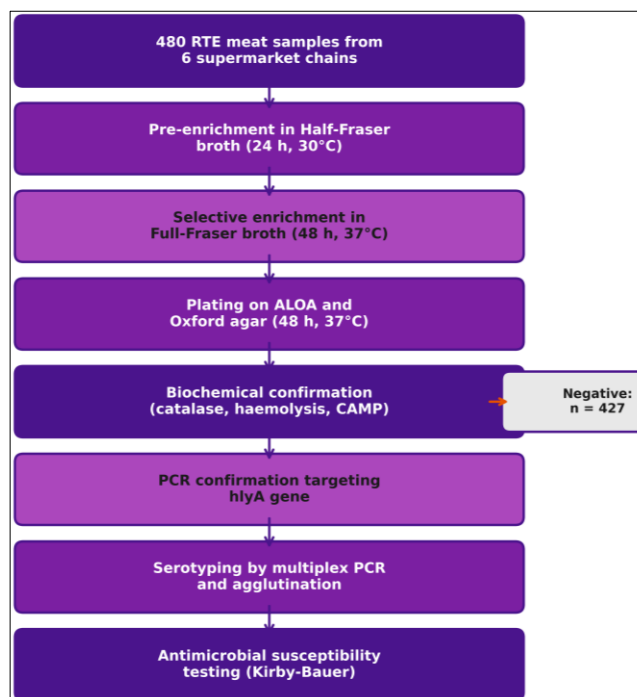
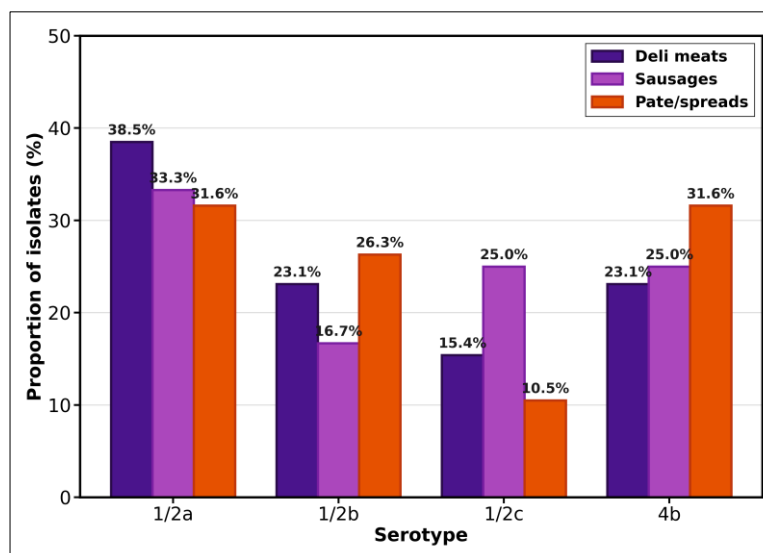


Fig 2: Antimicrobial resistance and intermediate susceptibility rates among 53 *L. monocytogenes* isolates tested against eight antibiotics by Kirby-Bauer disc diffusion. Values inside bars indicate the resistant fraction (%)

Table 2: Temporal distribution of Multi-Drug Resistant (MDR) isolates across four quarterly periods

Period	Total isolates	MDR isolates	MDR rate (%)	Median resistance classes
Q1 (Jan-Mar)	9	1	11.1	1.7
Q2 (Apr-Jun)	12	2	16.7	2.0
Q3 (Jul-Sep)	21	7	33.3	2.8
Q4 (Oct-Dec)	11	3	27.3	2.3

**Fig 3:** Schematic workflow for isolation, confirmation, serotyping, and antimicrobial susceptibility testing of *L. monocytogenes* from RTE meat samples. Of 480 samples processed, 53 yielded confirmed positive isolates and 427 were negative at the biochemical confirmation stage**Fig 4:** Distribution of *L. monocytogenes* serotypes across the three RTE meat product categories. Serotype 1/2a was the most frequent overall, while serotype 4b was proportionally highest in pate/spread products

Comprehensive interpretation

These results reveal a persistent, seasonally modulated presence of *L. monocytogenes* in RTE meat products at the retail level in Johannesburg, six years after the 2017-2018 outbreak prompted regulatory reform. The overall 11.0% prevalence sits close to the Sub-Saharan African pooled estimate of 12.8% and exceeds the EU benchmark of below 5% for RTE products at retail. Pate and spreads carry the greatest risk, likely because their soft, high-moisture matrix and neutral pH create conditions that favour *Listeria* multiplication during

refrigerated storage. The winter peak aligns with longer product residence times on shelves (lower consumer turnover of cold meats in cooler weather) and potential lapses in cold-chain management when ambient temperatures drop and retailers may reduce refrigerator intensity to save energy. The presence of first-line antibiotic resistance in over 10% of isolates, and MDR in nearly a quarter, suggests that food-chain contamination may also be contributing to the dissemination of resistant *Listeria* strains into the community.

Discussion

The principal findings of this research can be stated concisely: 11.0% of RTE meat products in Johannesburg supermarkets harboured *L. monocytogenes*, pate products were the most contaminated category, contamination followed a clear winter seasonal peak, and roughly one in four isolates was multi-drug resistant.

These prevalence figures are consistent with published data from comparable settings. Mhone *et al.* [6] reported a pooled African RTE meat prevalence of 12.8%, and a 2022 survey of Pretoria delicatessen counters found 9.1% positivity for *Listeria* spp. though only 6.3% for *L. monocytogenes* specifically [15]. European data tend to be lower; the EFSA 2021 zoonoses report documented a 2.4% prevalence in RTE meat at retail across EU member states [16]. The gap suggests that while South African regulations have tightened since 2018, implementation at the retail level still lags behind European standards.

Where the present data add something new is in the seasonal dimension. Most African prevalence studies are cross-sectional, capturing a single snapshot. The twelve-month sampling scheme used here revealed a 3.8-fold variation between the lowest month (April, 5.0%) and the highest (August, 19.2%). This pattern runs counter to the naive expectation that warmer months would favour bacterial growth; instead, it mirrors findings from temperate European countries [12, 13] and suggests that the cold-chain and shelf-life dynamics of winter retailing are more important drivers than ambient temperature per se.

Possible explanations for the winter peak include reduced consumer purchase frequency of cold meats in cooler weather, which extends the time products spend on display shelves approaching their "use by" dates; lower foot traffic in store aisles leading to less frequent restocking and potentially longer open-door periods for display refrigerators; and the well-documented ability of *L. monocytogenes* to multiply, albeit slowly, at the 4-8 degrees Celsius range typical of retail display cabinets [3].

The antimicrobial resistance data raise separate concerns. Ciprofloxacin resistance at 41.8% was the highest observed rate, a finding that aligns with growing reports of fluoroquinolone-resistant *Listeria* from food sources in East and Southern Africa [10, 17]. Ciprofloxacin is not a first-line treatment for listeriosis, so the direct clinical effect may be limited, but it signals selective pressure from the widespread veterinary and agricultural use of fluoroquinolones in the region. More troubling is the 12.3% penicillin resistance and 9.8% ampicillin resistance, because the standard empirical treatment for invasive listeriosis is intravenous ampicillin combined with gentamicin [18]. With gentamicin resistance at 28.7%, the possibility of encountering isolates resistant to both first-line agents simultaneously is no longer theoretical.

The MDR rate of 24.5% exceeds what has been reported in most European surveys (typically 5-15%) [11] but falls within the range observed in other African countries, suggesting that the selective pressures driving multi-drug resistance operate broadly across the continent's food production systems.

Limitations

Several limitations merit acknowledgement. The research was confined to the Johannesburg metropolitan area and may not reflect conditions in rural or peri-urban retail settings. Product sampling was limited to pre-packaged items from supermarket chains and did not include open-counter delicatessen products, which may carry different risk profiles. Serotyping was

performed by multiplex PCR and agglutination, which do not provide the resolution of whole-genome sequencing for outbreak tracking. And the twelve-month sampling window, while capturing seasonal trends, represents a single annual cycle; inter-annual variability was not assessed.

Conclusion

This research set out to measure the prevalence, serotype distribution, and antimicrobial resistance of *L. monocytogenes* in RTE meat products from supermarket chains in Johannesburg over a full calendar year. The investigation was motivated by the ongoing need for retail-level surveillance in South Africa following the devastating 2017-2018 listeriosis outbreak.

The key findings paint a concerning picture. One in nine RTE meat products (11.0%) tested positive for *L. monocytogenes*, with pate and spread products reaching 15.0% prevalence. A strong winter seasonal peak brought contamination rates above 19% in August, suggesting that cold-chain management during cooler months deserves targeted attention from both retailers and regulators. Serotype 1/2a dominated the isolate collection, consistent with its status as the most common food-associated serotype globally, while the clinically aggressive serotype 4b accounted for over a quarter of isolates. Antimicrobial resistance to ciprofloxacin (41.8%), tetracycline (34.2%), and gentamicin (28.7%) was widespread, and one in four isolates qualified as multi-drug resistant.

From a practical standpoint, these results support several recommendations. Retailers should audit cold-chain temperatures in RTE display cases during winter months, when the combination of slower product turnover and psychrotrophic *Listeria* growth creates an elevated hazard window. Pate and meat spread products, as the highest-risk category, may benefit from shorter shelf-life assignments or enhanced listericidal treatments such as high-pressure processing. Regulatory authorities should consider expanding routine retail surveillance to include antimicrobial resistance monitoring, which would provide early warning of emerging MDR clones entering the consumer food supply.

Future research should extend this work geographically to include smaller cities and rural retail environments, where cold-chain infrastructure may be less reliable. Whole-genome sequencing of retail isolates, linked to clinical isolates from listeriosis cases, would enable source attribution and track the persistence of specific clones across the production-to-retail continuum. Longitudinal studies spanning multiple years would clarify whether the winter contamination peak is a consistent feature or subject to inter-annual variation driven by weather, consumer behaviour, or changes in retail practice. And cost-benefit analyses of interventions such as active packaging with antimicrobial films, UV-C light treatment of display case surfaces, and real-time temperature monitoring with automated alerts would help retailers prioritise investments in *Listeria* control.

Acknowledgements

Institutional support

Johannesburg Metropolitan University provided access to its BSL-2 food microbiology laboratory, PCR suite, and cold-storage facilities for the duration of the research.

Contributions not qualifying for authorship

The authors thank the laboratory technicians who assisted with sample processing and the graduate students who participated in the monthly collection rotations.

Funding sources

This research was funded by the South African National Research Foundation through a Thuthuka Grant for emerging researchers in food safety.

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How to Cite This Article

Molefe T. Prevalence and antimicrobial resistance of *Listeria monocytogenes* in ready-to-eat meat products from supermarket chains. *International Journal of Veterinary Sciences and Animal Husbandry*. 2025; 10(10): 68-73.

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