



Research Paper

Antimicrobial Resistance Profiles of *Listeria* Species Recovered from Retail Outlets in Gauteng Province, South Africa



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ABSTRACT

The study determined the antimicrobial resistance (AMR) profiles of *Listeria* spp. (*L. monocytogenes*, *L. innocua*, and *L. welshimeri*) recovered from beef and beef products sold at retail outlets in Gauteng Province, South Africa. A total of 112 isolates of *Listeria* spp., including *L. monocytogenes* (37), *L. innocua* (65), and *L. welshimeri* (10), were recovered from beef and beef products collected from 48 retail outlets. *Listeria* spp. was recovered by direct selective plating following selective enrichment, and PCR was used to confirm and characterize recovered isolates. The disc diffusion method determined the resistance to 16 antimicrobial agents. All 112 isolates of *Listeria* spp. exhibited resistance to one or more antibiotics ($P < 0.05$). The prevalence of AMR in *Listeria* isolates was high for nalidixic acid (99.1%) and cefotaxime (80.4%) but low for gentamycin (2.7%), sulfamethoxazole-trimethoprim (3.6%), azithromycin (5.4%), and doxycycline (6.3%). Overall, for the three species of *Listeria*, the prevalence of resistance varied significantly only for streptomycin ($P = 0.016$) and tetracycline ($P = 0.034$). Multidrug-resistant isolates were detected in 75.7% (28/37), 61.5% (40/65), and 80% (8/10) isolates of *L. monocytogenes*, *L. innocua*, and *L. welshimeri*, respectively. The prevalence of AMR was significantly affected by the location and size of retail outlets, type of beef and beef products, and serogroups of *L. monocytogenes*. The high prevalence of AMR, particularly among the *L. monocytogenes* isolates, poses potential therapeutic implications for human consumers of contaminated beef products. There is, therefore, a need to regulate and enforce the use of antimicrobial agents in humans and animals in South Africa.

The genus *Listeria* consists of 17 recognized species: small rod, non-spore-forming gram-positive, facultative, anaerobic coccobacilli (Den Bakker et al., 2014). Only two species, *Listeria monocytogenes* and *L. ivanovi*, are considered pathogenic (Alexander et al., 1992; Koopmans et al., 2023). *Listeria monocytogenes* is the primary human foodborne pathogen and the third leading cause of mortality due to foodborne diseases in the USA (Scallan et al., 2015). Additionally, in immunocompromised individuals, *L. innocua* has been established as the causative agent of human listeriosis (Favaro et al., 2014; Perrin et al., 2003). Human listeriosis cases and outbreaks caused by *L. monocytogenes* have a considerable negative economic impact on society and the food industry (Ivanek et al., 2009). The food industry mostly uses the identification of *Listeria* species as a marker for the presence and growth of *L. monocytogenes* (Orsi and Wiedmann, 2016). Over the

years, medicine has been confronted with the advancement of highly resistant bacterial strains, which, given their worldwide distribution, are of international concern (Nolte 2014). Antimicrobial resistance has become a public health concern, not only because of inadequate treatment choices but also because of the economic problems posed to the globe (Nolte, 2014).

Varying frequency of contamination of meat products sampled at retail outlets for *Listeria* spp. resistant to one or more antimicrobials has been documented in Spain and Turkey (Arslan & Baytur, 2019; Gómez et al., 2014). These findings indicate that improperly cooked beef and beef products may serve as sources of listeriosis (Arslan & Baytur, 2019; Santorum, Garcia, Lopez, & Martínez-Suárez, 2012; Thomas et al., 2020).

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In South Africa, the increased threat of AMR has compelled the development of five strategic frameworks for national plans, which include the early detection and optimization of surveillance of AMR for reporting national, regional, and local resistance patterns of targeted antimicrobial choices (DAFF, 2018, OIE, 2016). However, as practiced in most developing countries, including South Africa, although regulations or laws exist regarding the types and use of antimicrobial agents in livestock, enforcing or controlling their use has always been difficult (Grace, 2015). Additionally, in response to the challenges faced by communal livestock farmers, which include high disease occurrence compounded by poor veterinary extension services (Mupfunya et al., 2021), the government of South Africa, through Fertilizers, Farm Feeds, Agricultural Remedies, and Stock Remedies Act 36 of 1947 legalized the over-the-counter availability of antimicrobial agents such as tetracyclines, sulfonamides, cloxacillin intramammary, fosfomycin, tylosin, and kitasamycin to facilitate timely treatment of easily recognizable endemic diseases. The implication is that using these antimicrobial agents as growth promoters for disease prevention and treatment in livestock is legal. These antimicrobial agents, particularly tetracyclines (Eagar et al., 2012; Mupfunya et al., 2021), are inexpensive and extensively used by livestock farmers in the country without veterinary oversight (Henton et al., 2011; Mupfunya et al., 2021; Naidoo, 2010; Theobald et al., 2019; Van et al., 2020). Unsurprisingly, this practice has led to livestock farmers' overuse or uncontrolled use of antimicrobial agents, thus causing increased resistance.

To date, there is no information on the antibiograms of *Listeria* spp., other than *L. monocytogenes*, nor are there publications on the distribution of antimicrobial-resistant (AMR) *Listeria* spp. in beef and beef products sold at retail outlets in Gauteng Province, South Africa. Therefore, the objectives of this study were to determine the antimicrobial resistance profiles of *L. monocytogenes*, *L. innocua*, and *L. welshimeri* to investigate the factors (external and internal) associated with the occurrence of AMR *Listeria* spp at retail outlets in Gauteng Province, South Africa. Finally, we assessed the distribution of AMR *L. monocytogenes* strains according to their serogroups. It is anticipated that the results of this study will contribute to addressing AMR challenges posed by *L. monocytogenes* in the country.

Materials and methods

Origin, isolation, and identification of *Listeria* species

The 112 isolates of *Listeria* spp. assayed for antimicrobial resistance in the current study originated from 400 samples of beef and beef products collected from retail outlets in Gauteng Province, South Africa (Gana et al., 2024). To isolate and identify *Listeria* spp. from processed samples, selective enrichment broth, isolation media (Matle et al., 2019), and multiplex polymerase chain reaction (mPCR) assays (Ryu et al., 2013) were used.

Simultaneous detection of the species of *Listeria* using multiplex polymerase chain reaction (PCR)

DNA extracts used as templates in the mPCR assays were prepared as described by Soumet et al. (1994). The primers used are shown in Supplementary Data, Table S1. PCR amplicons were electrophoresed on a 3% agarose gel using 1 × Tris-acetate-EDTA (TAE) buffer and stained with ethidium bromide. *L. monocytogenes* ATCC 19111, *Listeria innocua* ATCC 33090, *L. welshimeri* ATCC 35897, *L. grayi* ATCC 25401, *L. ivanovii* ATCC 19119, and *L. seeligeri* ATCC 35967 were used as the positive controls. *Campylobacter fetus* ATCC 27373 was used as a negative control, and water was used as a blank.

Determination of the serogroups of *L. monocytogenes* isolates

The mPCR assay was used to characterize *L. monocytogenes* isolates based on their serogroups, as earlier described (Doumith et al., 2004). The five primers used to classify the strains into serogroups are shown in Supplementary Data, Table S2. The five primers used to serotype *L. monocytogenes* are provided in Supplementary Data Table S2, and target genes *imo1118*, *imo0737*, *orf2110*, *orf2819*, and *prs* (genus-specific control). *L. monocytogenes* ATCC 19111 was used as the positive control. The products were electrophoresed on a 3% agarose gel, and a gel documentation system (Vacutec, South Africa) was used to capture amplicon sizes.

Selection of antimicrobial agents used and determination of the resistance of *Listeria* isolates to antimicrobial agents

The 16 antimicrobial agents belonged to eight antimicrobial classes: Beta-lactam, Cephalosporins, Aminoglycosides, Tetracyclines, Fluoroquinolones, Macrolides, Lincosamides, and Sulfonamides. To determine the resistance of *Listeria* spp. to 16 antimicrobial agents among the isolates of *Listeria* spp., the Kirby-Bauer disk diffusion method (Gautam et al., 2013) and the interpretation criteria recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018) were used.

For this study, the selected 16 antimicrobial agents were based on their ease of availability to livestock farmers, their use by veterinary and medical practitioners, and feedback received following consultations with veterinarians in Gauteng Province, South Africa. The types and disc concentrations of antimicrobial agents used were as follows: penicillin (10 units), amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), cephalothin (30 µg), cefotaxime (30 µg), streptomycin (25 µg), gentamicin (10 µg), kanamycin (30 µg), tetracycline (30 µg), doxycycline (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg), azithromycin (15 µg), clindamycin (10 µg), and sulfamethoxazole-trimethoprim (23.75/1.25 µg).

For antimicrobial agents for which the cut-off values for susceptibility were not stated for *Listeria*, the values provided for staphylococci were used as recommended by Conter et al. (2009). The following strains were used as controls: *L. monocytogenes* ATCC 19111, *Listeria innocua* ATCC 33090, *L. welshimeri* ATCC 35897, and *Campylobacter fetus* ATCC 273737373. For this study, any isolate that exhibited intermediate (I) or resistance (R) was classified as resistant to an antimicrobial agent.

Statistical analyses of data

The data were analyzed using the Statistical Package for Social Science (version 25, IBM) to determine whether there were statistically significant differences in the frequency of isolation of antimicrobial-resistant *Listeria* spp. (the independent variable) and dependent variables, specifically, the geographical location (district) of retail outlets, the size of retail outlets, types of beef and beef products, and serogroups of *L. monocytogenes*. Data are presented as frequencies (percentages), and any association was determined using chi-square and Fisher's exact tests with the level of significance set at an alpha level of 0.05.

Results

Prevalence of AMR in *L. monocytogenes*, *L. innocua*, and *L. welshimeri* isolates recovered from retail outlets

Overall, 111 (99.1%) of 112 isolates of *Listeria* exhibited resistance to one or more of the 16 antimicrobial agents tested, and the prevalence was low for gentamycin (2.7%) and sulfamethoxazole-

Table 1 Prevalence of resistance to antimicrobial agents exhibited according to the species of *Listeria* recovered from beef and beef products

<i>Listeria</i> species	No. of isolates tested ^a	No. (%) of isolates resistant	No. (%) of isolates resistant to															
			CEP ^b	K	GEN	S	TE	DOX	NA	CIP	ENR	AZI	CLIN	P	AMC	AMP	SXT	
<i>L. monocytogenes</i>	37	37 (100.0)	29 (78.4)	2 (5.4)	1 (2.7)	10 (27.0)	8 (26.2)	1 (2.7)	37 (100.0)	4 (10.8)	7 (18.9)	2 (5.4)	23 (62.2)	4 (10.8)	4 (10.8)	2 (5.4)		
<i>L. innocua</i>	65	64 (98.5)	53 (81.5)	9 (13.8)	1 (1.5)	33 (50.8)	27 (41.5)	6 (9.2)	64 (98.5)	5 (7.7)	5 (7.7)	3 (4.6)	43 (66.2)	3 (4.6)	3 (4.6)	1 (1.5)		
<i>L. welshimeri</i>	10	10 (100.0)	8 (80.0)	2 (20.0)	1 (10.0)	7 (70.0)	1 (10.0)	0 (0.0)	10 (100.0)	0 (0.0)	1 (10.0)	1 (10.0)	9 (90.0)	1 (10.0)	1 (10.0)	0 (0.0)		
<i>p</i> value		1	0.928	0.303	0.304	0.016	0.034	1	1	1	0.234	0.718	0.246	0.296	0.472	0.289		
Total	112	111 (99.1)	90 (80.4)	13 (11.6)	3 (2.7)	50 (44.6)	36 (32.1)	7 (6.3)	111 (99.1)	10 (8.9)	13 (11.6)	6 (5.4)	75 (67.0)	8 (7.1)	8 (7.1)	4 (3.6)		

^a CEP: Cefotaxime, CEP: Cephalothin, K: Kanamycin, GEN: Gentamicin, S: Streptomycin, TE: Tetracycline, DOX: Doxycycline, NA: Nalidixic acid, CIP: Ciprofloxacin, ENR: Enrofloxacin, AZI: Azithromycin, CLIN: Clindamycin, P: Penicillin, AMC: Amoxicillin-clavulanic acid, AMP: Ampicillin, SXT: Sulfamethoxazole-Trimethoprim.

trimethoprim (SXT) (3.6%) (Table 1). However, a comparatively high prevalence of resistance was detected for cefotaxime (80.4%) and nalidixic acid (99.1%). The differences were statistically significant ($P < 0.05$). A similar pattern was observed for all three species of *Listeria*. Among the three species of *Listeria*, differences in the prevalence of resistance to antimicrobial agents varied significantly only for streptomycin ($P = 0.016$) and tetracycline ($P = 0.034$).

All (100%) 37 isolates of *L. monocytogenes* tested exhibited resistance to one or more of the antimicrobial agents, and resistance was displayed to 16 antimicrobial agents. The prevalence of resistance was low, 2.7% (1/37), for gentamicin and doxycycline, but high for nalidixic acid, 100% (37/37), and cefotaxime, 78.4% (29/37). The differences were statistically significant ($P < 0.05$).

Among the 65 *L. innocua* isolates, 64 (98.5%) exhibited resistance to one or more antimicrobial agents. The prevalence of resistance was low for gentamicin and sulfamethoxazole-trimethoprim (SXT), 1.5% (1/65), and amoxicillin-clavulanic acid and ampicillin, 4.6% (3/65), but high for cefotaxime, 81.5% (53/65), and nalidixic acid, 98.5% (64/65). The differences were statistically significant ($P < 0.05$).

For the 10 isolates of *L. welshimeri*, resistance was exhibited to 13 antimicrobial agents. The range of resistance varied from 10% (1/10) to gentamicin, tetracycline, enrofloxacin, azithromycin, amoxicillin-clavulanic acid, and ampicillin, but was high to cefotaxime, 80% (8/10), clindamycin, 90% (9/10), and nalidixic acid, 100% (10/10) ($P < 0.05$).

Table 1 compares the frequency of resistance exhibited by the three species of *Listeria* to 16 antimicrobial agents.

Geographical location of retail outlets and its relationship to antimicrobial resistance in Listeria spp

The geographical location of the retail outlets significantly affected the prevalence of resistance to four antimicrobial agents in *L. monocytogenes* for cefotaxime ($P < 0.001$), cephalothin ($P = 0.019$), streptomycin ($P = 0.028$), and penicillin ($P = 0.038$) (Table 2). For the 65 isolates of *L. innocua*, the geographical location of the outlets significantly affected the prevalence of resistance to the following eight antimicrobials: CEF ($P < 0.001$), kanamycin ($P = 0.047$), streptomycin ($P = 0.001$), tetracycline ($P = 0.021$), doxycycline ($P = 0.032$), nalidixic acid ($P < 0.001$), clindamycin ($P < 0.001$), and penicillin ($P = 0.002$). Among the 10 *L. welshimeri* isolates, the location of the retail outlets significantly affected the prevalence of five antimicrobial agents: cefotaxime ($P = 0.022$), streptomycin ($P = 0.026$), nalidixic acid ($P = 0.016$), clindamycin ($P = 0.020$), and penicillin ($P = 0.045$).

Prevalence of AMR in L. monocytogenes, L. innocua, and L. welshimeri isolates recovered by the size of retail outlets

Table 3 shows the distribution of AMR *Listeria* spp. according to the size of the retail outlets where the beef and beef products originated.

Of the 37 isolates of *L. monocytogenes*, the size of the retail outlet (chain, large, medium, and small) had a statistically significant effect on the prevalence of five antimicrobial agents, namely, cefotaxime ($P = 0.001$), cephalothin ($P = 0.009$), ciprofloxacin ($P = 0.005$), clindamycin ($P = 0.011$), and penicillin ($P = 0.004$).

Concerning the 65 isolates of *L. innocua*, the prevalence of resistance to antimicrobial agents varied significantly across the four sizes of outlets for six antimicrobial agents: cefotaxime ($P < 0.001$), streptomycin ($P = 0.001$), tetracycline ($P = 0.008$), doxycycline ($P = 0.013$), clindamycin ($P = 0.004$), and penicillin ($P = 0.002$). For the 10 *L. welshimeri* isolates, the size of the retail outlets significantly affected the prevalence of resistance to four antimicrobial agents: cefotaxime ($P = 0.002$), streptomycin ($P = 0.006$), clindamycin ($P = 0.002$), and penicillin ($P = 0.021$). Across the three spe-

Table 2
Prevalence of antimicrobial resistance in *L. monocytogenes*, *L. innocua*, and *L. welshimeri* according to the geographical location of the retail outlets.

Variable	No. of isolates of <i>L. monocytogenes</i> (n = 37)	No. (%) of isolates resistant to															
		^a CEF	CEP	K	GEN	S	TE	DOX	NA	CIP	ENR	AZI	CLIN	P	AMC	AMP	SXT
Region																	
Pretoria North	10	8 (80.0)	2 (20.0)	0 (0.0)	0 (0.0)	4 (40.0)	3 (30.0)	0 (0.0)	10 (100.0)	0 (0.0)	1 (10.0)	2 (20.0)	8 (80.0)	6 (60.0)	0 (0.0)	0 (0.0)	1 (10.0)
Pretoria East	10	7 (70.0)	2 (20.0)	1 (10.0)	0 (0.0)	2 (20.0)	2 (20.0)	0 (0.0)	10 (100.0)	10 (100.0)	3 (30.0)	0 (0.0)	4 (40.0)	3 (30.0)	0 (0.0)	1 (10.0)	1 (10.0)
Pretoria West	9	7 (77.8)	2 (22.2)	0 (0.0)	1 (11.1)	2 (22.2)	2 (22.2)	1 (11.1)	9 (100.0)	2 (22.2)	1 (11.1)	0 (0.0)	9 (100.0)	7 (77.8)	3 (33.3)	2 (22.2)	0 (0.0)
Pretoria South	1	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pretoria Central	7	6 (85.7)	1 (14.3)	1 (14.3)	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)	7 (100.0)	1 (14.3)	2 (28.6)	0 (0.0)	2 (28.6)	7 (100.0)	1 (14.3)	1 (14.3)	0 (0.0)
<i>p</i> value		<0.001	0.019	0.187	0.374	0.028	0.078	0.374	1	0.218	0.051	0.374	0.051	0.038	0.220	0.095	0.178
Region																	
Pretoria North	10	8 (80.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (50.0)	4 (40.0)	1 (10.0)	10 (100.0)	0 (0.0)	10 (100.0)	0 (0.0)	6 (60.0)	4 (40.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pretoria East	15	15 (100.0)	4 (26.7)	4 (26.7)	1 (6.7)	4 (26.7)	4 (26.7)	1 (6.7)	14 (93.3)	3 (20.0)	3 (20.0)	2 (13.3)	9 (60.0)	13 (86.7)	2 (13.3)	2 (13.3)	0 (0.0)
Pretoria West	12	10 (83.3)	0 (0.0)	1 (8.3)	0 (0.0)	5 (41.7)	11 (91.7)	2 (16.7)	12 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	11 (91.7)	9 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pretoria South	18	13 (72.2)	3 (16.7)	2 (11.1)	0 (0.0)	11 (61.1)	6 (33.3)	2 (11.1)	18 (100.0)	0 (0.0)	0 (0.0)	1 (5.5)	10 (55.6)	13 (72.2)	1 (5.5)	1 (5.5)	1 (5.5)
Pretoria Central	10	7 (70.0)	0 (0.0)	2 (20.0)	0 (0.0)	5 (50.0)	3 (30.0)	0 (0.0)	10 (100.0)	2 (20.0)	10 (100.0)	0 (0.0)	7 (70.0)	10 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>p</i> value		<0.001	0.193	0.047	0.374	0.001	0.021	0.032	<0.001	0.178	0.130	0.223	<0.001	0.002	0.223	0.223	0.374
Region																	
Pretoria North	2	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pretoria East	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pretoria West	2	2 (100.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (100.0)	1 (50.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pretoria South	3	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Pretoria Central	3	2 (66.7)	2 (66.7)	2 (66.7)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	1 (33.3)	1 (33.3)	3 (100.0)	3 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)
<i>p</i> value		0.022	0.09	0.374	0.374	0.026	0.374	NA	0.016	NA	0.374	0.374	0.020	0.045	0.374	0.374	NA

^a CEF: Cefotaxime, CEP: Cephalothin, K: Kanamycin. GEN: Gentamicin, S: Streptomycin, TE: Tetracycline, DOX: Doxycycline. NA: Nalidixic acid, CIP: Ciprofloxacin, ENR: Enrofloxacin, AZI: Azithromycin.

Table 3
Antimicrobial resistance of isolates of *L. monocytogenes*, *L. innocua*, and *L. welshimeri* from retail outlets to antimicrobial agents by the size of the outlet.

Size of outlet ^a	No. of isolates of <i>L. monocytogenes</i> (n = 37)	No. (%) of isolates resistant to:																p value
		^a CEF	CEP	E	GEN	S	TE	DOX	NA	CIP	ENR	AZI	CLIN	P	AMC	AMP	SXT	
Chain	12	10 (83.3)	2 (16.7)	0 (0.0)	1 (8.3)	4 (33.3)	5 (41.7)	0 (0.0)	12 (100.0)	1 (8.3)	2 (16.7)	1 (8.3)	6 (50.0)	6 (50.0)	0 (0.0)	2 (16.7)	0 (0.0)	0.003
Large	7	4 (57.1)	2 (28.6)	0 (0.0)	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)	7 (100.0)	1 (14.3)	2 (28.6)	1 (14.3)	4 (57.1)	6 (85.7)	3 (42.9)	2 (28.6)	0 (0.0)	0.001
Medium	7	6 (85.7)	1 (14.3)	1 (14.3)	0 (0.0)	2 (28.6)	1 (14.3)	1 (14.3)	7 (100.0)	1 (14.3)	0 (0.0)	0 (0.0)	7 (100.0)	4 (57.1)	0 (0.0)	0 (0.0)	0 (0.0)	0.009
Small	11	9 (81.8)	2 (18.2)	1 (9.1)	0 (0.0)	3 (27.3)	1 (9.1)	0 (0.0)	11 (100.0)	1 (9.1)	3 (27.3)	0 (0.0)	6 (54.5)	7 (63.6)	0 (0.0)	0 (0.0)	2 (18.2)	0.005
p value		0.001	0.009	0.197	0.391	0.990	0.166	0.391	NA ^b	0.005	0.071	0.203	0.011	0.004	0.391	0.203	0.391	
Size of outlet ^a	No. of isolate of <i>L. innocua</i> (n = 65)	No. (%) of isolates resistant to:																p value
		CEF	CEP	E	GEN	S	TE	DOX	NA	CIP	ENR	AZI	CLIN	P	AMC	AMP	SXT	
Chain	15	12 (80.0)	0 (0.0)	1 (6.7)	0(0.0)	9 (60.0)	8 (53.3)	2 (13.3)	15 (100.0)	2 (13.3)	4 (26.7)	2 (13.3)	11 (73.3)	13 (86.7)	0 (0.0)	1 (6.7)	0(0.0)	0.002
Large	19	16 (84.2)	2 (10.5)	1 (5.3)	0 (0.0)	8 (42.1)	5 (26.3)	1 (5.3)	18 (94.7)	1 (5.3)	0 (0.0)	1 (5.3)	11 (57.9)	14 (73.7)	2 (10.5)	1 (5.3)	0(0.0)	0.006
Medium	18	15 (83.3)	3 (16.7)	5 (27.8)	1 (5.6)	10 (55.6)	10 (55.6)	2 (11.1)	18 (100.0)	5 (27.8)	1 (5.6)	0(0.0)	13 (72.2)	14 (77.8)	0 (0.0)	0(0.0)	0(0.0)	0.002
Small	13	10 (76.9)	2 (15.4)	2 (15.4)	0(0.0)	6 (46.2)	5 (38.5)	1 (7.7)	18 (100.0)	0 (0.0)	0 (0.0)	0(0.0)	8 (61.5)	7 (53.8)	1 (7.7)	1(7.7)	1(7.7)	0.003
p value		<0.001	0.067	0.076	0.391	0.001	0.008	0.013	NA	0.151	0.293	0.236	0.0004	0.002	0.189	0.064	0.391	
Size of outlet ^a	No. of isolates of <i>L. welshimeri</i> (n = 10)	No. (%) of isolates resistant to:																p value
		CEF	CEP	K	GEN	S	TE	DOX	NA	CIP	ENR	AZI	CLIN	P	AMC	AMP	SXT	
Chain	1	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.0004
Large	4	3 (75.0)	1 (25.0)	1 (25.0)	0] (0.0)	3 (75.0)	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	0 (0.0)	0(0.0)	4 (100.0)	3 (75.0)	1 (25.0)	1 (25.0)	0 (0.0)	0.004
Medium	3	2 (66.7)	0(0.0)	0(0.0)	0(0.0)	2 (66.7)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	0(0.0)	2 (66.7)	1 (33.3)	0 (0.0)	0(0.0)	0 (0.0)	0.028
1 (50.0)	2	2 (100.0)	1 (50.0)	0(0.0)	0(0.0)	1 (50.0)	1 (50.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	1 (50.0)	0 (0.0)	0(0.0)	0 (0.0)	0.007
p value		0.002	0.132	0.278	0.391	0.006	0.391	NA	NA	NA	0.391	0.391	0.002	0.021	0.391	0.391	NA	

^a Chain: Retailers with more than one outlet, Large: 5–6 outlets, Medium: 3–5 outlets, and Small: 1–2 outlets.

^b CEP: Cephalothin, CEF: Cefotaxime, K: Kanamycin, GEN: Gentamicin, S: Streptomycin, TE: Tetracycline, DOX: Doxycycline, NA: Nalidixic acid, CIP: Ciprofloxacin, ENR: Enrofloxacin, ZI: Azithromycin, CLIN: Clindamycin, P: Penicillin, AMC: Amoxicillin-clavulanic acid, AMP: Ampicillin, SXT: Sulfamethoxazole-Trimethoprim, bNA: Not applicable.

Table 4
Antimicrobial resistance of *L. monocytogenes*, *L. innocua*, and *L. welschimeri* isolates obtained from retail outlets by type of beef and beef products.

Type of product ^a	No. of isolates <i>L. monocytogenes</i> tested (n = 37)	No. (%) of isolates resistant to:																P value
		CEF	CEP	K	GEN	S	TE	DOX	NA	CIP	ENR	AZI	CLIN	P	AMC	AMP	SXT	
Biltong	2	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.05
Minced beef	16	14 (87.5)	0 (0.0)	1 (6.3)	0 (0.0)	7 (43.8)	2 (12.5)	0 (0.0)	16 (100.0)	1 (6.3)	2 (12.5)	2 (12.5)	10 (62.5)	9 (56.3)	0 (0.0)	0 (0.0)	1 (6.3)	0.008
Raw beef	12	9 (75.0)	6 (50.0)	1 (8.3)	0 (0.0)	3 (25.0)	3 (25.0)	0 (0.0)	12 (100.0)	2 (16.7)	4 (33.3)	0 (0.0)	7 (58.3)	8 (66.7)	2 (16.7)	3 (25.0)	1 (8.3)	0.0007
Cold beef	7	5 (71.4)	1 (14.3)	0 (0.0)	1 (14.3)	0 (0.0)	1 (14.3)	1 (14.3)	7 (100.0)	1 (14.3)	1 (14.3)	0 (0.0)	5 (71.4)	6 (85.7)	2 (28.6)	1 (14.3)	0 (0.0)	0.004
p value		0.003	0.266	0.187	0.391	0.205	0.167	0.391	NA	0.092	0.117	0.391	0.0009	0.066	0.203	0.204	0.187	
Type of product ^a	No. of isolates <i>L. innocua</i> tested (n = 65)	No. (%) of isolates resistant to:																P value
		CEF	CEP	K	GEN	S	TE	DOX	NA	CIP	ENR	AZI	CLIN	P	AMC	AMP	SXT	
Beef patties	5	5 (100.0)	0 (0.0)	1 (20.0)	0 (0.0)	3 (60.0)	1 (20.0)	1 (20.0)	5 (100.0)	1 (20.0)	2 (40.0)	1 (20.0)	3 (60.0)	3 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.001
Biltong	7	6 (85.7)	2 (28.6)	1 (14.3)	0 (0.0)	6 (85.7)	2 (28.6)	0 (0.0)	7 (100.0)	0 (0.0)	1 (14.3)	0 (0.0)	6 (85.7)	4 (57.1)	1 (14.3)	1 (14.3)	0 (0.0)	0.003
Minced beef	21	20 (95.2)	5 (23.8)	4 (19.0)	0 (0.0)	12 (57.1)	7 (33.3)	2 (9.5)	21 (100.0)	4 (19.0)	2 (9.5)	2 (9.5)	14 (66.7)	20 (95.2)	2 (9.5)	1 (4.8)	0 (0.0)	0.002
Raw beef	22	15 (68.2)	0 (0.0)	2 (9.1)	0 (0.0)	9 (40.9)	14 (63.6)	2 (9.1)	22 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	11 (50.0)	16 (72.7)	0 (0.0)	0 (0.0)	1 (4.5)	0.008
Cold beef	10	7 (70.0)	0 (0.0)	1 (10.0)	1 (10.0)	3 (30.0)	4 (40.0)	1 (10.0)	9 (90.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (90.0)	6 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.008
p value		0.000	0.180	0.003	0.374	0.004	0.007	0.037	NA	0.178	0.158	0.212	0.000	0.001	0.189	0.123	0.374	
Type of product ^a	No. of isolates <i>L. welschimeri</i> tested (n = 10)	No. (%) of isolates resistant to:																P value
		CEF	CEP	K	GEN	S	TE	DOX	NA	CIP	ENR	AZI	CLIN	P	AMC	AMP	SXT	
Biltong	1	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.083
Minced beef	2	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.048
Raw beef	7	5 (71.4)	3 (42.9)	2 (28.6)	1 (14.3)	5 (71.4)	1 (14.3)	0 (0.0)	7 (100.0)	0 (0.0)	1 (14.3)	1 (14.3)	7 (100.0)	6 (85.7)	1 (14.3)	1 (14.3)	0 (0.0)	0.001
p value		0.011		0.423	0.423		0.423	^b NA	NA	NA	0.423	0.423		0.423	0.423	0.423	NA	

^a CEF: Cefotaxime, CEP: Cephalothin, K: Kanamycin. GEN: Gentamycin, S: Streptomycin, TE: Tetracycline, DOX: Doxycycline. NA: Nalidixic acid, CIP: Ciprofloxacin, ENR: Enrofloxacin, AZI: Azithromycin, CLIN: Clindamycin, P: Penicillin, AMC: Amoxicillin-clavulanic acid, AMP: Ampicillin, SXT: Sulfamethoxazole-Trimethoprim.

^b NA: Not applicable.

Table 5
Detection of the same antimicrobial resistance patterns within and between species of *Listeria* isolated from retail outlets

Antimicrobial-resistant pattern	Species of <i>Listeria</i>			p value
	<i>L. monocytogenes</i>	<i>L. innocua</i>	<i>L. welshimeri</i>	
No. of isolates	37	65	10	
No. (%) of patterns detected per species	31 (83.8)	43 (66.2)	8 (80.0)	0.136
No. (%) of MDR patterns ^a	28 (75.7)	40 (61.5)	8 (80.0)	0.234
No. (%) of species-specific patterns	23 (62.2)	34 (52.3)	3 (30.0)	0.185
No. (%) and types of patterns shared between LM* and LI	5 (16.1) ^b	5 (11.6) ^b	ND ^c	0.559
	^d P-CEF-NA	P-CEF-NA		
	P-CEF-S-CLIN-NA-AZI-TE	P-CEF-S-CLIN-NA-AZI-TE		
	P-CEF-S-CLIN-NA	P-CEF-S-CLIN-NA		
	P-CEF-CLIN-NA-TE	P-CEF-CLIN-NA-TE		
	P-CEF-CEP-S-K-CLIN-NA	P-CEF-CEP-S-K-CLIN-NA		
No. (%) and types of patterns shared between LM and LW	1 (3.2) ^e		1 (12.5) ^e	0.289
	CEF-CLIN-NA	ND	CEF-CLIN-NA	
No. (%) and types of patterns shared between LI and LW	ND	2 (4.7) ^f	2 (25.0) ^f	0.049
		P-S-CLIN-NA	P-S-CLIN-NA	
		CEF-S-NA	CEF-S-NA	
No. (%) and types of patterns shared among LM, LI, and LW	2 (6.5) ^g	2 (4.7) ^g	2 (25.0) ^g	0.124
	P-CEF-S-CLIN-NA	P-CEF-S-CLIN-NA	P-CEF-S-CLIN-NA	
	CEF-S-CLIN-NA	CEF-S-CLIN-NA	CEF-S-CLIN-NA	

^a MDR: Patterns consisting of three or more antimicrobial agents.
^b The five patterns were detected in 7 (22.6%) of 31 and 14 (32.6%) of 43 patterns exhibited by *L. monocytogenes* and *L. innocua*, respectively.
^c ND: Not detected.
^d P: Penicillin, NA: Nalidixic acid; P: CEF: Cefotaxime, CLIN: Clindamycin, S: Streptomycin, CIP: Ciprofloxacin, TE: Tetracycline, AZI: Azithromycin
^e The resistance pattern was detected in 2 (6.5%) of 31 and 2 (25.0%) of 8 patterns exhibited by *L. monocytogenes* and *L. welshimeri*, respectively.
^f The two resistance patterns were detected in 2 (4.7%) of 43 and 2 (25.0%) of 8 patterns exhibited by *L. innocua* and *L. welshimeri*, respectively.
^g The two resistance patterns were detected in 2 (6.5%) of 31, 7 (16.3%) of 43, and 3 (37.5%) of 8 patterns exhibited by *L. monocytogenes*, *L. innocua*, and *L. welshimeri*, respectively.

Table 6
Prevalence of resistance to antimicrobial agents by *L. monocytogenes*, *L. innocua*, and *L. welshimeri* by class of agents

Antimicrobial Class (8)	Type of antimicrobial Agent	No. (%) of isolates that exhibited resistance ^a to antimicrobial agents in class:			
		<i>L. monocytogenes</i> (n = 37)	<i>L. innocua</i> (n = 65)	<i>L. welshimeri</i> (n = 10)	P value
Aminoglycoside (3)	Gentamicin, Streptomycin, Kanamycin	10 (27.0)	33 (50.8)	7 (70.0)	0.016
Beta lactams (4)	Amoxicillin-clavulanic acid, Penicillin, Ampicillin	23 (62.2)	49 (75.4)	6 (60.0)	0.296
Fluoroquinolones (3)	Ciprofloxacin, Nalidixic acid, Enrofloxacin	37 (100.0)	64 (98.5)	10 (100.0)	1
Tetracycline (2)	Doxycycline, Tetracycline	8 (21.6)	27 (41.5)	1 (10.0)	0.034
Cephalosporin (1)	Cephalothin, Cefotaxime	29 (78.4)	53 (81.5)	8 (80.0)	0.928
Lincosamide (1)	Clindamycin	23 (62.2)	43 (66.2)	9 (90.0)	0.246
Macrolides (1)	Azithromycin	2 (5.4)	3 (4.6)	1 (10.0)	0.780
Sulfonamides (1)	Sulfamethoxazole-trimethoprim	2 (5.4)	1 (1.5)	0 (0.0)	1
					Total (n = 112)

(): Number of antimicrobial agents in the class.
^a Resistant to any of the antimicrobial agents in the class.

cies of *Listeria*, the size of the retail outlet significantly affected the prevalence of cefotaxime ($P = 0.002$), streptomycin ($P = 0.006$), clindamycin ($P = 0.002$), and penicillin ($P = 0.021$). Overall, within each of the four sizes of retail outlets, the difference in antimicrobial resistance across the 16 antimicrobial agents for the three species of *Listeria* varied significantly ($P < 0.05$).

Prevalence of AMR in L. monocytogenes, L. innocua, and L. welshimeri isolates recovered from retail outlets according to the types of beef products

The prevalence of resistance exhibited by *Listeria* spp. according to the type of beef and beef product shown in Table 4. Among the 37 isolates of *L. monocytogenes* recovered from the four types of beef products, statistically significant differences were detected in the prevalence of resistance to two antimicrobial agents, namely cefo-

taxime ($P = 0.003$) and clindamycin ($P = 0.009$). For the nine isolates of *L. monocytogenes* recovered from RTE foods (biltong: 2 isolates and cold beef: 7 isolates), resistance was displayed to 12 antimicrobial agents at the following prevalence: cefotaxime (66.7%), clindamycin (66.7%), nalidixic acid (100%), tetracycline (33.3%), amoxicillin-clavulanic acid (22.2%), and 11.1% (cephalothin, gentamicin, doxycycline, ciprofloxacin, enrofloxacin, penicillin, and ampicillin).

For the 65 isolates of *L. innocua*, across the five products tested, statistically different resistance was exhibited to seven antimicrobials, namely cefotaxime ($P = 0.000$), kanamycin ($P = 0.003$), streptomycin ($P = 0.004$), tetracycline ($P = 0.007$), doxycycline ($P = 0.037$), clindamycin ($P = 0.000$), and penicillin ($P = 0.001$). Among the 17 *L. innocua* obtained from RTE foods (biltong:7, cold beef:10), resistance was exhibited to 12 antimicrobials tested: cefotaxime (70.6%), kanamycin (11.8%), gentamicin (5.9%), strepto-

Table 7
Antimicrobial resistance of *L. monocytogenes* isolates obtained from farms, abattoirs, and retail outlets according to their serogroups

Serogroup	No. (%) of isolates from retail outlets are resistant to:														P value		
	CEF ^a	CEP	K	GEN	S	TE	DOX	NA ^b	CIP	ENR	AZI	CLIN	P	AMC		AMP	SXT
1/2a-3a	12 (75.0)	4 (25.0)	1 (6.3)	1 (6.3)	4 (25.0)	2 (12.5)	0 (0.0)	16 (100.0)	0 (0.0)	2 (12.5)	0 (0.0)	11 (68.8)	9 (56.3)	3 (18.8)	3 (18.8)	2 (12.5)	0.002
1/2c-3c	2	1	0	0	1	0	2	0	0	1	1	1	2	0	0	0	0.003
4b-4d-4e	16 (84.2)	2 (10.5)	1 (5.3)	0 (0.0)	5 (26.3)	6 (31.6)	1 (5.3)	19 (100.0)	1 (5.3)	4 (21.1)	1 (5.3)	11 (57.9)	12 (63.2)	1 (5.3)	1 (5.3)	0 (0.0)	0.005
<i>p</i> value	0.677	0.284	1	0.457	0.751	1	1	1	1	0.418	1	0.753	0.678	1	1	0.202	

^a CEF: Cefotaxime, CEP: Cephalothin, K: Kanamycin, GEN: Gentamicin, S: Streptomycin, TE: Tetracycline, DOX: Doxycycline, NA: Nalidixic acid, CIP: Ciprofloxacin, ENR: Enrofloxacin, AZI: Azithromycin, CLIN: Clindamycin, P: Penicillin, AMC: Amoxicillin-clavulanic acid, AMP: Ampicillin, SXT: Sulfamethoxazole-Trimethoprim.
^b NA: Not applicable.

mycin (35.3%), tetracycline (29.4%), doxycycline (11.8%), nalidixic acid (82.4%), ciprofloxacin (5.9%), enrofloxacin (11.8%), azithromycin (5.9%), clindamycin (70.6%), and penicillin (52.9%).

Among the ten isolates of *L. welshimeri* recovered from the three types of beef and beef products, the prevalence of resistance varied significantly for cefotaxime ($P = 0.011$) and streptomycin ($P = 0.036$) (Table 4). Biltong was the only RTE food that yielded *L. welshimeri* ($n = 1$); it was resistant to cefotaxime, streptomycin, and nalidixic acid.

The outlet sources of *Listeria* spp. did not significantly affect the prevalence of antimicrobial resistance ($P > 0.05$; $n = 48$) (data not shown).

Resistance patterns exhibited by isolates of Listeria

The types and frequencies of the resistance patterns detected for the three species of *Listeria* isolated from retail outlets are shown in Supplementary Data, Table S3. A total of 70 resistance patterns were exhibited by all isolates of *L. monocytogenes*, *L. innocua*, and *L. welshimeri*, singly or in combination. The number and frequency of antimicrobial agents in each resistance pattern were as follows: 1 (2, 2.9%), 2 (4, 5.7%), 3 (14, 20%), 4 (14, 20%), 5 (11, 15.7%), 6 (8, 11.4%), 7 (8, 11.4%), 8 (5, 7.1%), 9 (2, 2.9%), and 10 (2, 2.9%). The differences were statistically significant ($P = 0.013$).

The frequencies of detection of resistance patterns were 83.8% (31/37), 66.2% (43/65), and 80% (8/10) among *L. monocytogenes*, *L. innocua*, and *L. welshimeri* isolates, respectively, but the differences were not statistically significant ($P = 0.136$) (Table 5). Resistance patterns unique to the three species were found in 23 (62.2%), 34 (52.3%), and 3 (30%) isolates of *L. monocytogenes*, *L. innocua*, and *L. welshimeri*, respectively ($P = 0.185$).

The occurrence of multidrug resistance (MDR) varied across the species of *Listeria*, 75.7%, 61.5%, and 80% in *L. monocytogenes*, *L. innocua* and *L. welshimeri*, respectively, but the differences were not statistically significant ($P = 0.234$). The patterns with the highest number of antimicrobial agents in the three species were P-AMP-CEP-CEF-S-CIP-ENR-CLIN-NA-TE (1 isolate, 10 antimicrobials) in *L. monocytogenes*, P-CEP-CEF-S-K-CIP-ENR-NA-TE (1 isolate, 9 antimicrobials) and P-AMC-AMP-CEP-CEF-S-K-CLIN-NA (1 isolate, 9 antimicrobials) in *L. innocua* and P-CEP-CEF-S-GEN-K-ENR-CLIN-NA-AZI (1 isolate, 10 antimicrobials) in *L. welshimeri* (Supplementary Data, Table S3).

The same five patterns were detected in *L. monocytogenes* and *L. innocua* (LM-LI), involving seven (22.6%) of 31 and 14 (32.6%) of 43, respectively. The same resistance pattern was detected between *L. monocytogenes* and *L. welshimeri* in two isolates each. Two isolates, each of *L. innocua* (4.7%, 2/43) and *L. welshimeri* (25%, 2/8), displayed the same resistance patterns, P-S-CLIN-NA and CEF-S-NA. The difference in the frequency was statistically significant ($P = 0.049$). Two resistance patterns, P-CEF-S-CLIN-NA and CEF-S-CLIN-NA, were detected in 2 isolates of *L. monocytogenes* (6.5%, 2/37), 7 isolates of *L. innocua* (16.3%, 7/43), and 3 isolates of *L. welshimeri* (37.5%, 3/8).

Prevalence of resistance to classes antimicrobial agents among L. monocytogenes, L. innocua, and L. welshimeri

The distribution of resistance by the three species of *Listeria* to antimicrobials in eight classes of antimicrobial agents is shown in Table 6. Overall, for the three species, the prevalence of resistance to antimicrobial classes was comparatively low for sulfonamides, 2.7% (3/112) and macrolides, 5.4% (6/112), moderate for lincosamide, 67% (75/112) and beta-lactam, 69.6% (78/112) and high to cephalosporin, 80.4% (90/112) and fluoroquinolones, 99.1% (111/112). Statistically significant differences were detected in the prevalence of resistance exhibited among the three species of *Listeria* for two antimicrobial classes, namely, aminoglycoside [($P = 0.016$;

Number of beef and beef products tested per region				
Pretoria North	Pretoria East	Pretoria West	Pretoria South	Pretoria Central
n=80	n=90	n=76	n=79	n=75
Number of <i>L. monocytogenes</i> isolates tested				
n=10	n=10	n=9	n=1	n=7
Distribution of type and number (%) of serogroups detected				
1/2a-3a: 2 (20.0)	1/2a-3a: 6 (60.0)	1/2a-3a: 7 (77.8)	1/2a-3a: 0 (0.0)	1/2a-3a: 1 (14.3)
1/2c-3c: 1 (10.0)	1/2c-3c: 1 (10.0)	1/2c-3c: 0 (0.0)	1/2c-3c: 0 (0.0)	1/2c-3c: 0 (0.0)
4b-4d-4e: 7 (70.0)	4b-4d-4e: 3 (30.0)	4b-4d-4e: 2 (22.2)	4b-4d-4e: 1 (100.0)	4b-4d-4e: 6 (85.7)
Frequency (%) of resistance exhibited to 16 antimicrobial agents				
10 (62.5)	12 (75.0)	13 (81.3)	3 (18.8)	11 (68.8)
Frequency (%) of multi-drug resistance (MDR)* among isolates				
9 (90.0)	7 (70.0)	9 (100.0)	1 (100.0)	7 (100.0)

*Overall, the frequency of MDR isolates of *L. monocytogenes* was 89.2% (33/37)

Figure 1. Frequency of detecting the serogroups and antimicrobial resistance in *L. monocytogenes* isolates according to the geographical location of the retail outlets.

range: *L. monocytogenes* (27%) – *L. welshimeri* (70%)] and tetracycline [$P = 0.034$; range: *L. welshimeri* (10%) – *L. innocua* (41.5%)].

The prevalence of resistance to the classes of antimicrobial agents among the isolates of *L. monocytogenes* was lowest compared to those exhibited by either *L. innocua* or *L. welshimeri* for aminoglycoside, cephalosporin, and lincosamide.

Prevalence of resistance to antimicrobial agents by serogroup of L. monocytogenes

There was no statistically significant relationship ($P > 0.05$) between resistance and serotype for the 16 tested antimicrobial agents (Table 7). Similarly, the frequencies of antimicrobial agents with the highest prevalence of resistance were 40% (6/15), 40% (6/15), and 20% (3/15), for serogroups 1/2a-3a, 1/2c-3c, and 1/4b-4d-4e, respectively ($P = 0.407$), except for NA, to which all the 37 isolates of *L. monocytogenes* exhibited resistance.

Among the 16 serogroup 1/2a-3a isolates, resistance to 13 antimicrobial agents was exhibited and was high to penicillin (56.3%), clindamycin (68.8%), cefotaxime (75%), and nalidixic acid (100%), but low at 6.3% (kanamycin and gentamycin) and 12.5% (tetracycline, enrofloxacin, and sulfamethoxazole-trimethoprim) ($P = 0.002$).

For the two isolates of serogroups 1/2c-3c, antimicrobial resistance was displayed to eight antimicrobial agents. The prevalence of resistance to cefotaxime (100%), nalidixic acid (100%), and penicillin (100%) was high but moderate (50%) for cephalothin, streptomycin, enrofloxacin, azithromycin, and clindamycin ($P = 0.003$).

Among the 19 isolates of serogroup 4b-4d-4e, resistance was exhibited to 14 antimicrobials and was high to cefotaxime (84.2%) and nalidixic acid (100%), moderate to clindamycin (57.9%), penicillin

(63.2%), but low (5.3%) for kanamycin, doxycycline, ciprofloxacin, azithromycin, amoxicillin-clavulanic acid, and penicillin ($P = 0.005$).

Distribution of antimicrobial resistance (AMR), multidrug resistance (MDR), and serogroups in L. monocytogenes isolates according to their geographical sources

The effect of the geographical location of the retails from where the *L. monocytogenes* isolates originated on the distribution of their serogroups and detecting antimicrobial resistance (AMR and MDR) are shown in Fig. 1. The 37 isolates of *L. monocytogenes* recovered from the five regions across Gauteng province were Pretoria North ($n = 10$), Pretoria East ($n = 10$), Pretoria West ($n = 9$), Pretoria ($n = 1$), and Pretoria Central ($n = 7$). The distribution of two serogroups varied significantly across the five regions as follows: 1/2a-3a ($p = 0.018$) and 4b-4d-4e ($p = 0.023$) but not for 1/2c-3c ($p = 1$). The frequency of resistance to the 16 antimicrobial agents varied significantly ($p = 0.002$), ranging from 18.8% (3/16) in Pretoria South to 75% (12/16) in Pretoria East. However, regardless of the regional sources of the *L. monocytogenes* isolates, the frequency of the *L. monocytogenes* isolates, the frequency of MDR was high (70%-100%), and the differences were not statistically significant ($p = 0.475$).

Discussion

Listeria spp., particularly *L. monocytogenes*, have been reported to be naturally susceptible to several antibiotics that target gram-positive bacteria (Troxler et al., 2000). The current study is the first documentation of the antibiograms of *L. innocua* and *L. welshimeri* from South

Africa and the first report to determine the factors associated with the contamination of beef and beef products with antimicrobial-resistant *L. monocytogenes* strains in the country. Matle et al. (2019) reported the prevalence of antimicrobial-resistant *L. monocytogenes* recovered from meat and meat products (cattle, poultry, sheep, pork, and game) collected from three major ports of entry (Durban, Cape Town, and Port Elizabeth) and retail outlets from nine provinces. The current study focuses on beef and beef products sampled at retail outlets from one province only (Gauteng province). Recently, Kayode and Okoh (2023) determined the prevalence of antimicrobial-resistant *Listeria monocytogenes* in RTE foods, including several nonmeat products in South Africa.

For the antimicrobials used in this study, resistance was observed against all 16 by at least some, but not all, isolates recovered from retail outlets. The prevalence of resistance was generally lower for gentamycin (2.7%), sulfamethoxazole-trimethoprim (3.6%), doxycycline (6.3%), and ampicillin (7.1%) but higher for clindamycin (67%), penicillin (69%), cefotaxime (80.4%), and nalidixic acid (99.1%). In an earlier study of meat and meat product samples at retail outlets from nine provinces in South Africa, Matle et al. (2019) reported that the highest resistance (100%) was observed against fosfomycin, colistin, and nalidixic acid, followed by streptomycin (99.0%), clindamycin (97.3%), gentamycin (74.4%), and sulfamethoxazole-trimethoprim (56.8%). On the other hand, Kayode and Okoh (2023), also in South Africa, reported a high prevalence of resistance to penicillin (75%), oxytetracycline (75%), and streptomycin (72.7%) but less resistance to gentamycin (17.4%), ampicillin (25%), and sulfamethoxazole-trimethoprim (34.8%) in *L. monocytogenes* recovered from RTE foods (including nonmeat products) sampled in the Eastern Cape province. The vast differences in the prevalence of antimicrobial resistance detected in *L. monocytogenes* among the three studies conducted in South Africa may be explained in part by the sample sources (Gauteng province versus East Cape province versus nine provinces), types of samples processed (beef and beef versus RTE foods versus meat and meat products from several livestock), types of antimicrobial agents tested, and the use of antimicrobial agents on the cattle farms from where the beef/meat products originated. The high prevalence of resistance to nalidixic acid across the three species of *Listeria* (98.5–100%) could be attributed to the fact that the strains of *Listeria* isolated were resistant to 25 mg/500 mL of enrichment, thus selecting for nalidixic-resistance *Listeria* that exhibited resistance to antimicrobial disc with nalidixic acid (30 µg).

Varying prevalence of the antimicrobial-resistant *L. monocytogenes* and other *Listeria* spp. have been documented in different countries. Unlike our study, where 100% and 98.4% of the isolates of *L. monocytogenes* and *L. innocua*, respectively, were resistant to one or more of the 16 antimicrobials tested, a considerably lower prevalence of 34.5% and 43.1% of the 20 antimicrobials tested in meat products and meat processing environments, respectively, in Spain was reported by Gómez et al. (2014) and resistance to clindamycin (90%) was found to be the most common resistance phenotype in *L. monocytogenes* (Escolar et al., 2017); in Turkey, 48.1% of *L. monocytogenes* isolates were reported to be resistant to three or more antimicrobials (Arslan & Baytur, 2019). These diverse findings on the prevalence of resistance to different types of antimicrobial agents outside South Africa may reflect differences in the types of antimicrobial agents, usage levels, and sample sources.

It is of epidemiological significance that our study found that the three variables investigated (regional location, size of retail outlets, and type of beef and beef products) had statistically significant effects on some of the antimicrobials tested. These differences may be multifactorial, including differences in the use of antimicrobial agents on cattle farms, practices during processing at abattoirs, size of retail outlets (chain, large, medium, and small) that may be impacted by the source of cattle slaughtered (feedlots, cow-calf, and communal farms), and finally, hygienic practices at retail outlets that may facilitate cross-

contamination by antimicrobial-resistant *L. monocytogenes*, such as the degree of handling during processing (raw beef versus minced beef). These factors have been shown to affect the occurrence of *L. monocytogenes* and antimicrobial-resistant *L. monocytogenes* in meat and meat products (Cheng et al., 2019; Kayode and Okoh, 2023; Matle et al., 2019; Noll et al., 2018; Seçil et al., 2012; Thompson, 2021).

It is of therapeutic relevance that the prevalence of resistance was similarly low for gentamycin, ampicillin, amoxicillin-clavulanic acid, and sulfamethoxazole-trimethoprim. Thus, the potential importance of these four antimicrobials as therapeutic options for consumers of beef and beef products contaminated by antimicrobial-resistant *Listeria* spp. and *Listeria* infections cannot be ignored (Obaidat, 2020). However, the high prevalence of penicillin resistance (69.1%) in *L. monocytogenes* isolates poses a significant concern, particularly therapeutic failure, because penicillin is a frontline antibiotic used to treat human listeriosis (Dos Reis et al., 2022). A considerably lower prevalence of resistance to some antimicrobials tested in our study has been reported. These include the reported AMR prevalence rates of 2%, 0%, and 0% for ciprofloxacin, penicillin, and tetracycline, respectively, in food products in Australia (Wilson et al., 2018) and a 1.6% prevalence of resistance to penicillin reported in Spain (Arslan & Baytur, 2019).

It is relevant to mention that tetracycline is the most used drug in the livestock industry in South Africa without veterinary oversight because it is classified as an over-the-counter drug, and it is inexpensive and readily available to livestock farmers. In the current study, 21.6% of *Listeria monocytogenes* isolates from beef and beef products exhibited resistance to the tetracycline class. It cannot be overemphasized that tetracycline is also used as a treatment option in some cases of human listeriosis, including treating patients who do not respond to standard therapy (Wilson et al., 2018).

The occurrence of MDR among the isolates of *Listeria* spp. poses potential therapeutic challenges, considering that among the 37 isolates of *L. monocytogenes*, 31 (83.3%) resistance patterns were detected, of which 28 (75.7%) were MDR, involving 3–10 antimicrobial agents. Notably, multidrug-resistant *L. monocytogenes* were resistant to penicillin, ampicillin, streptomycin, and tetracycline, which are optional treatments for human listeriosis. These findings reflect the overuse of antimicrobial agents in the cattle industry due to their legal use as growth promoters and the prevention and treatment of bacterial diseases without veterinary oversight (Henton et al., 2011; Naidoo, 2010). Both species of *Listeria* displayed a similar resistance pattern to these antimicrobials. Others have reported varying frequencies of detection of resistance patterns, MDR, and antimicrobial resistance exhibited by the isolates of *Listeria* spp. recovered from meat and meat products in South Africa (Kayode and Okoh, 2023; Matle et al., 2019), Germany (Noll et al., 2018), Turkey (Şanlıbaba et al., 2020), and Spain (Escolar et al., 2017; Gómez et al., 2014).

The epidemiological, food safety, and public health significance of the documented presence of *L. monocytogenes* and *L. innocua* in the same food niche have been highlighted based on the potential transfer of genetic materials (resistance and virulence genes) between both species. It is pertinent to mention that although *Listeria monocytogenes* is the most pathogenic *Listeria* species (Allam et al., 2018; Koopmans et al., 2023), and *L. innocua* is generally considered nonpathogenic, but it has been associated with listeriosis in immunocompromised humans (Favaro et al., 2014; Perrin et al., 2003). Our study provides some evidence and potential for the transfer of resistance between *L. monocytogenes* and *L. innocua* with the demonstration that seven isolates of *L. monocytogenes* and 14 isolates of *L. innocua* exhibited the same five resistance patterns: P-CEF-NA, P-CEF-S-CLIN--NA-AZI-TE, P-CEF-S-CLIN-NA, P-CEF-CLIN-NA-TE, and P-CEF-CEP-S-K-CLIN-NA. In addition, two resistance patterns, P-CEF-S-CLIN-NA and CEF-S-CLIN-NA, were found in two isolates: *L. monocytogenes*, *L. innocua*, and *L. welshimeri*. These observations of the same resistance patterns

between *L. monocytogenes* and *L. innocua* may be due to coinfection by both organisms in cattle and exposure to similar antimicrobial agents either in feeds or during therapy of the cattle reared on farms or cross-contamination during processing at abattoirs or handling at retail outlets. Our findings could also be due to the transfer or sharing of antimicrobial-resistant genetic materials due to chromosomal mutations or acquired genes harbored on transposons or plasmids (Baquero et al., 2020). Therefore, the potential to transfer resistance genes from nonpathogenic *Listeria* species to pathogenic *L. monocytogenes* exists (Li et al., 2022), and the suggestion that *L. innocua* can form a reservoir of resistance genes that may transfer between bacterial species, including pathogens, raises some public health concerns (Gómez et al., 2014).

Although we did not detect statistically significant ($P > 0.05$) differences in the prevalence of resistance among the three serogroups (1/2a–3a, 1/2c–3c, and 1/4b–4d–4e) of *L. monocytogenes* to the 16 antimicrobials tested, it is of therapeutic importance that these three serogroups have been associated with human listeriosis (Wu et al., 2016). However, Matle et al. (2019) have found that the isolates of *L. monocytogenes* in serogroup 4b–4d–4e were resistant to penicillin G, gentamicin, and meropenem. In contrast, the authors reported that all 1/2c–3c serogroup isolates were resistant to streptomycin, penicillin G, and serogroup 1/2a–3a were resistant to streptomycin, clindamycin, fusidic acid, and nitrofurantoin. The differences in the findings of the studies conducted in South Africa may be attributed to the sources of the isolates assessed in both studies, including those originating from local and foreign sources. A therapeutically relevant finding in our research is that within each of the three serogroups, *L. monocytogenes* isolates exhibited resistance to antimicrobials reported to be used as options to treat human listeriosis (Olaimat et al., 2018). In the most recent outbreak of human listeriosis in South Africa, considered the largest in the world to date, serogroup 4b–4d–4e of *L. monocytogenes* was implicated (Allam et al., 2018). Studies by others have reported the diversity and antimicrobial resistance profiles of *L. monocytogenes* serogroups isolated from various food products (Şanlıbaba et al., 2020; Wieczorek and Osek, 2017).

In addition to our study demonstrating the effects of the size of retail outlets and types of beef and beef products significantly affecting the prevalence of resistance to some antimicrobial agents in both *L. monocytogenes* and other *Listeria* spp., our study also brings to light a fascinating aspect regarding the significant effect of the geographical locations of the retail outlets from which the 37 isolates of *L. monocytogenes* originated on the distribution of their serogroups (1/2a–3a and 4b–4d–4e) and the resistance to some antimicrobial agents. This finding adds to the existing body of knowledge on the effect of geographical sources of *L. monocytogenes* on the frequency of serogroups, exhibition of phenotypic resistance, and carriage of resistance genes (Gana et al., 2024; Matle et al., 2019; Noll et al., 2018; Yina et al., 2015).

The disc diffusion method, along with the CLSI (Conter et al., 2009) criteria used in the current study, has been applied to determine the prevalence of resistance in *L. monocytogenes* and other species of *Listeria* by others (Lotfollahi et al., 2017). However, some limitations have been identified with this method, including its subjective nature and, more importantly, the fact that the cut-off values for the zones of inhibition, although available for some antimicrobial agents (penicillin, ampicillin, and sulfamethoxazole) (CLSI, 2018) are not listed for others. Therefore, as reported by others (Conter et al., 2009), the cut-off values provided for staphylococci were used, which is a limitation of this study.

Conclusion

The generally high prevalence of resistance to antimicrobial agents and the exhibition of MDR among *Listeria monocytogenes* and *L. innocua*

isolates recovered from beef and beef products to the most used antimicrobial agents in the country have therapeutic and food safety implications. Beef and beef products sold at retail outlets contaminated with antimicrobial-resistant pathogenic serogroups of *L. monocytogenes* may enter the human food chain, causing listeriosis with potential therapeutic failure. Considering that most antimicrobial agents used in the livestock industry in South Africa are readily available over the counter for farmers and the fact that AMR genes can be transferred from nonpathogenic *L. innocua* to pathogenic *L. monocytogenes* strains, it is prudent to implement effective control of the use of antimicrobial agents in the country. Future studies should use molecular methods such as pulse-field gel electrophoresis (PFGE) and whole genome sequencing (WGS) to confirm the genetic relatedness of *Listeria* spp., which exhibited the same antimicrobial resistance patterns.

Declaration of competing interest

The authors declare no conflicts of interest. The funders or the project did not participate in the study design, the collection of samples, or the analysis or interpretation of data. Furthermore, the funders played no role in the decision to publish the results of our investigation.

CRediT authorship contribution statement

James Gana: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Nomakorinte Gcebe:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Rebone Moerane:** Visualization, Supervision, Resources, Project administration, Funding acquisition. **Yusuf Ngoshe:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation. **Takula Tshuma:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation. **Khomotso Moabelo:** Writing – original draft, Methodology, Investigation. **Abiodun Adegisiyun:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Approval by the Ethics Committees

Before the commencement of the study, the research protocol was approved by the following committees, namely, the Research Ethics Committee (REC) of the Faculty of Veterinary Science, University of Pretoria, South Africa (REC 138-19), Animal Ethics Committee (AEC) of the Faculty of Veterinary Science, University of Pretoria, South Africa (REC 138-19), and Section 20 according to Act 35 of 1984 by the Director of Animal Health at the Department of Agricul-

ture, Forestry and Fisheries (DAFF), [Number:12/11/1/1/8(1131)] South Africa.

Appendix A. Supplementary material

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.jfp.2024.100322>.

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