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SHORT COMMUNICATION

Whole genome characterization of *Listeria welshimeri* isolates recovered from cattle abattoirs and retailers in Gauteng province in South Africa

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Abstract
The study used whole-genome sequencing (WGS) to characterize 12 isolates of *L. welshimeri* recovered from the beef production chain in Gauteng province, South Africa. Two sequence types (STs) were identified, with ST3294 accounting for 75% (9/12) and ST1084 for 16.7% (2/12) of the isolates. Antimicrobial resistance (AMR) plasmids detected included conjugative plasmid (MOBP2; T_virB11; virb4) in 58.3% and AMR plasmid (NFO33156) in 41.7% of the isolates. Two AMR genes, *fosX* (25%) and *vga (G) (*100%), along with 11 virulence factors, were present in all 12 isolates of *L. welshimeri*. The phylogenomic tree of *L. welshimeri* clustering based on the ST according to the source, type, and class of beef and beef products revealed three groups: ST3294 ($n=9$), ST1084 ($n=2$), and unassigned ST ($n=1$). The findings of this study demonstrate the potential for transferring genetic materials from non-pathogenic *L. welshimeri* to pathogenic *L. monocytogene*s within the same ecological niche.

Keywords Whole genome sequencing · *Listeria welshimeri* · Beef production chain · Antimicrobial resistance genes · Virulence factors · Phylogeny · South Africa

1 Introduction

Globally, *Listeria monocytogenes* (*L. monocytogenes*) is the most frequent cause of human listeriosis, with the world's largest documented outbreak occurring in South Africa due to the consumption of contamination of a ready-to-eat meat product (Allam et al. 2018; Koopmans et al. 2023). Although *L. innocua* and *L. welshimeri* are generally considered non-pathogenic, they have been implicated in listeriosis cases among immunocompromised humans.

Most post-outbreak studies in South Africa have focused on detecting *L. monocytogenes* in meat and meat products.

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Recent genomic studies have documented the presence of *L. innocua* and *L. monocytogenes* isolates in various levels of the beef production chain, including cattle farms, abattoirs, and retail outlets in Gauteng province (Gana et al. 2023, 2024). The current study used WGS to characterize *L. welshimeri* isolates and compare their genomic profiles with those of *L. innocua* and *L. monocytogenes* recovered from the same sources in the beef production chain in Gauteng province.

2 Results and discussion

Our earlier study determined the prevalence of *L. welshimeri* in the beef production chain in South Africa as follows: 0% (0/328) on cattle farms, 0.8% (2/262) in cattle abattoirs and 2.5% (10/400) in retail outlets (Gana 2022). Subsequently, we reported the genomic sequences of *L. innocua* (Gana et al. 2023) and *L. monocytogenes* (Gana et al. 2024) isolates recovered from the same 3 sources, but not for *L. welshimeri*.

Table 1 shows the frequency of STs, conjugative plasmids, AMR plasmids, AMR genes, and virulence factors among the *L. welshimeri* isolates recovered from the beef production chain in Gauteng province. The detection frequency of STs among the 12 *L. welshimeri* isolates was: ST3294 (75%), ST1084 (16.7%), and unassigned ST (8.3%) ($p<0.001$). Compared to previosly published data on *L. innocua* and *L. monocytogenes* isolates recovered from the same sources (Gana et al. 2023, 2024), the most frequently detected of the 14 STs in 110 *L. innocua* isolates were ST637 (26.4%), ST448 (20%) and ST1085 (12.7%) (Gana et al. 2023), while in the 7 STs identified in the 60 isolates of *L. monocytogenes*, ST204 (46.7%) and ST2 (21.7%) were predominant (Gana et al. 2024). All the STs identified in the 3 species of *Listeria* (*L. welshimeri*, *L. innocua*, and *L. monocytogenes)* were species-specific. In contrast to our findings, Mafuna et al. (2022), detected ST1005, ST1084, and ST168 in *L. welshimeri* isolates recovered from meat and food processing facilities in South Africa. The diversity of STs within and between *Listeria* species has been attributed differences. For example, ST1050 and ST2331 were prevalent in the *L. welshimeri* isolated from food processing environments in Russia (Voronina et al. 2023).

One conjugative plasmid (MOBP2; T_virB11; virb4) was detected in 58.3% (7/12) of *L. welshimeri* isolates; the distribution frequency by sample source was 50% (5/10) in retail outlets and 100% (2/2) in abattoirs ($p=0.470$), while by ST, the frequency was 85.7% (6/7) for ST3294 and 50% (1/2) for ST1084 ($p=0.284$). However, compared to *L. monocytogenes* isolates from the same sources (Gana et al. 2024), 80% (48/60) harboured conjugative plasmids comprising 3 plasmids (MOBP2, MOBV, and FA_orf13; FA_orf17b). The conjugative plasmids detected in *L. welshimeri* in the current study were associated with their STs, as found in *L. monocytogenes* recovered from the same sample source. Our findings agree with the study's report in China that demonstrated that a conjugative plasmid in *L. monocytogenes*, pLM1686, was associated with 4 STs (ST87, ST59, ST9, and ST120) and that the carriage of the plasmid provides the pathogen the advantage to survive in an adverse environment.

One AMR plasmid (NF033156) was detected in 41.7% (5/12) of the *L. welshimeri* isolates, with a distribution of 50% (1/2) in abattoir isolates and 40% (4/10) in retail outlet isolates ($p>0.793$). In contrast, the same AMR plasmid (NF033156) was detected in only 5% (3/60) of *L. monocytogenes* isolates (Gana et al. 2024). The significantly higher prevalence of AMR plasmid (NF033156) in *L. welshimeri* compared to *L. monocytogenes* in both studies may be attributed to differences in species and environmental sources of the isolates. Mafuna et al. (2022) detected only plasmid pLGUG1 in *L. welshimeri*, while *L. innocua* isolates recovered from meat and food processing facilities in South Africa carried plasmid J1776. The plasmid-associated AMR gene NF033156 is associated with the Bleomycin binding protein (BLMA) family. This protein family constitutes proteins related to glyoxalase I and ring-opening dioxygenases. These proteins can bind bleomycin or related antibiotics, which could assist with export and confer resistance when transferred from producer strains to other strains of *Listeria*. Such mobile genetic elements have been shown to typically cause a diverse functional variation in the *Listeria* accessory genome (Palaiodimou et al. 2021).

Only 2 AMR genes were detected: *fosX*, which encodes Fosfomycin resistance, was found in 25% of *L. welshimeri* isolates, while *vga(G)*, an ABC-F type ribosomal protection protein conferring lincosamide resistance, was found in all *L. welshimeri* isolates. The 3 *L. welshimeri* isolates positive for the *fosX* resistance gene were distributed in 2 isolates of ST1084 and an unidentified ST. In contrast, all 110 *L. innocua* isolates carried both the *fosX* and *lin* (encoding phenotype-lincosamide) (Gana et al. 2023). Similarly, all 60 isolates of *L. monocytogenes* carried both genes [*fosX* and *vga(G)*] (Gana et al. 2024). In this study, all isolates of *L. welshimeri* ($n=12$), *L. innocua* ($n=110$), and *L. monocytogenes* ($n=60$) recovered from the same sources (Gana et al. 2023, 2024) carried phenotypic Fosfomycin-encoding resistance genes and lincosamide-encoding resistance genes, showing that these resistance genes are widespread in *Listeria* spp. across the beef production chain in Gauteng province. This could be partly due to the frequent use of Fosfomycin and lincosamide in the country's livestock industry and the transfer of AMR genes and AMR plasmids across species of *Listeria* in the beef production chain. The transfer of resistance genes

Table 1 Frequency of ST, conjugative genes, AMR plasmid, AMR gene, and virulence factors in *L. welshimeri* isolates from abattoirs and beef and beef products from retailers

SampleID	Source	Type (Class)	ST	Plasmid		Chromosome												
				Conjugative gene ($n=1$)	AMR gene ($n=1$)	AMR ($n=2$)		VF ($n=11$)										
						fosX	vga(G)	clpC	clpE	clpP	fbpA	hbp1/svpA	hbp2	Lap	lpeA	lplA1	lspA	prsA2
CFSAN 119,112	Cattle abattoir (High throughput)	Environmental-effluent	3294	MOBP2; T_virB11; virb4	NF033156	neg*	pos	pos**	pos	pos	Pos	pos	pos	pos	pos	pos	pos	pos
CFSAN 119,114	Cattle abattoir (High throughput)	Environmental-effluent	3294	MOBP2; T_virB11; virb4	ND	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CFSAN 117,543	Retail outlet (Chain)	Beef steak (Raw)	3294	ND	ND	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CFSAN 117,463	Retail outlet (Large)	Spleen (Raw)	1084	ND	ND	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CFSAN 117,499	Retail outlet (Large)	Wors (Raw)	3294	MOBP2; T_virB11; virb4	NF033156	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CFSAN 117,548	Retail outlet (Large)	Cheese beef (Raw)	ND	ND	ND	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CFSAN 119,145	Retail outlet (Large)	Beef short rib (Raw)	3294	MOBP2; T_virB11; virb4	ND	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CFSAN 117,468	Retail outlet (Medium)	Beef stew (Raw)	3294	ND	ND	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CFSAN 117,532	Retail outlet (Medium)	Biltong' (RTE)	1084	MOBP2; T_virB11; virb4	NF033156	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CFSAN 117,533	Retail outlet (Medium)	Minced beef (Raw)	3294	MOBP2; T_virB11; virb4	NF033156	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CFSAN 117,522	Retail outlet (Small)	Minced beef (Raw)	3294	ND	ND	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CFSAN 119,140	Retail outlet (Small)	Beef sheen (Raw)	3294	MOBP2; T_virB11; virb4	NF033156	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos

ND: Not detected

*neg: negative

**pos: positive

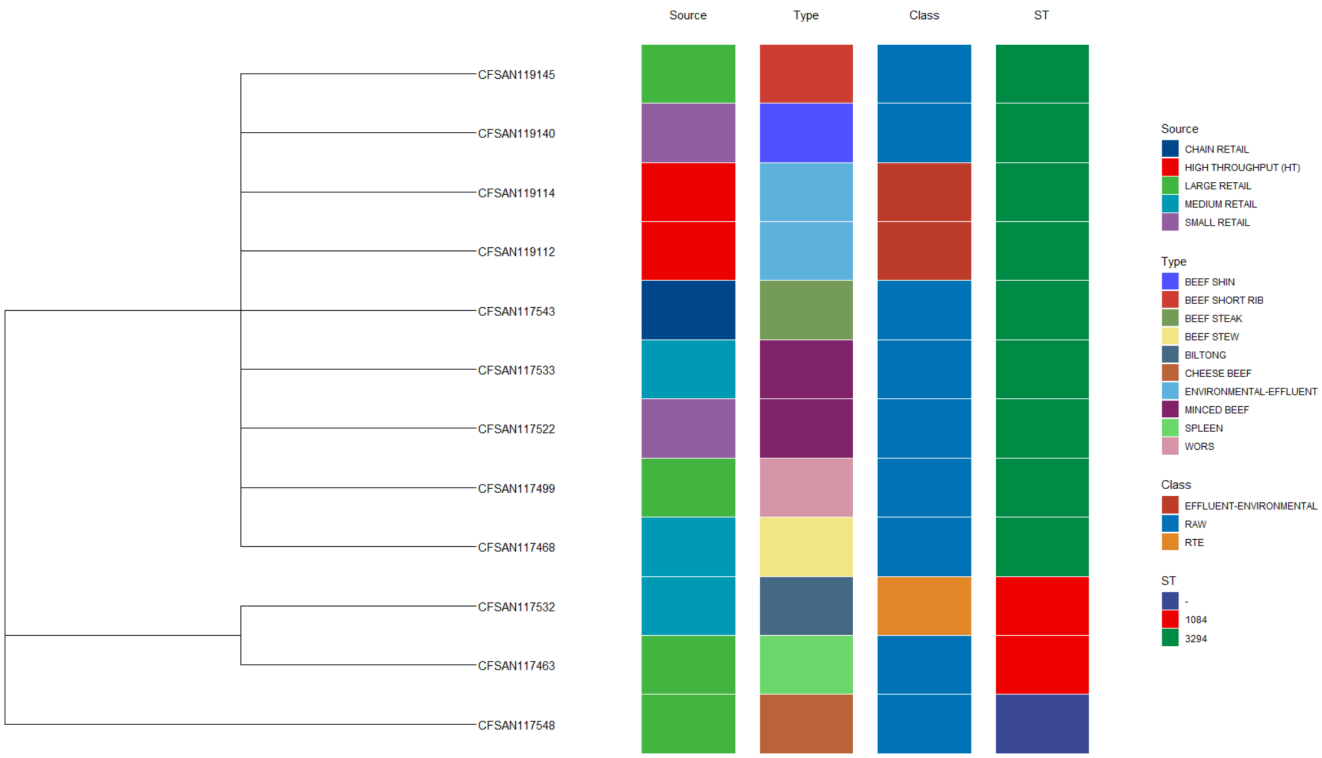

Fig. 1 Phylogenomic tree of *L. welshimeri* detected across the cattle abattoirs and retail outlets. The first colour legend indicates the source, the second the type of sample, the third represents the class of beef and beef products, and the fourth the STs (3094 and 1084) (Color figure online)

from non-pathogenic to pathogenic *Listeria* and other bacterial pathogens may have therapeutic implications. Interestingly, different AMR genes were detected in *L. welshimeri* isolates recovered from environmental waters in the Eastern Cape province, South Africa, carrying 6 AMR genes (*sull*, *tetA*, *blaTEM*, *blaSHV*, *blaOXA-1*, and *blaCIT*) (Mpondo et al. 2021). The difference between both studies may be due to the sources of *L. welshimeri* in our study (cattle farms, abattoirs, and retail outlets) versus environmental waters.

All 12 *L. welshimeri* isolates carried 11 virulence factors detected in this study. Ten of these factors (*clpC*, *clpE*, *clpP*, *hbp1/svpA*, *hbp2*, *lap*, *IpeA*, *lplA1*, *lspA* and *prsA2*) were also identified in *L. innocua* and *L. monocytogenes* recovered from the same sources in the beef production chain in Gauteng province (Gana et al. 2023, 2024). The potential for virulence factor transfer among the 3 *Listeria* species cannot be ignored. However, 15 virulence factors unique to 44 virulence factors detected in the 60 *L. monocytogenes* isolates, including *hly*, *prfA*, *hpt*, *inlB*, *inlC*, *lntA*, *plcA*, and *plcB*, recovered from the 3 levels of the chain, have been demonstrated to play an important role in the pathogenesis of the organism (Koopmans et al. 2023). On the other hand, these virulence factors were undetected in the 12 and 110 isolates of *L. welshimeri* and *L. innocua*, respectively, which may explain their lower pathogenicity. Nonetheless, both species have been implicated in listeriosis cases among immunocompromised humans (Favaro et al. 2014). Some virulence factors detected in *L. welshimeri* isolates play essential roles and functions in *L. monocytogenes* pathogenesis, including facilitating adhesion (*fbpA* and *Iap*) and invasion (*IpeA*).

The phylogenomic tree of *L. welshimeri* displayed clear clustering based on ST: ST3294 ($n=9$), ST1084 ($n=2$), and unassigned ST ($n=1$) (Fig. 1). The distribution of *L. welshimeri* isolates across product source, type, and class categories suggest an indistinct grouping pattern, indicating a product-independent distribution of *L. welshimeri* isolates within the beef production chain.

3 Conclusion

This study demonstrates the potential for genetic material transfer from non-pathogenic *L. welshimeri* to pathogenic *L. monocytogenes* within the same ecological niche. The detection of the same conjugative plasmid (MOBP2), AMR plasmid (NF033156), AMR genes (*fosX* and *vga(G)/lin*), and 10 virulence factors in *L. welshimeri*, *L. innocua*, and *L. monocytogenes* isolates across the beef production chain in Gauteng province could have epidemiologic, food safety and therapeutic implications for humans. Further research

is needed to understand the role of the virulence factors detected in *L. welshimeri* isolates.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00003-025-01546-8 .

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Data availability All samples have been deposited under NCBI BioProject PRJNA215355 and can be searched based on the isolate CFSAN identifier.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Institutional review board statement The study was approved and conducted under terms approved by the Research Committee (*REC 138–19*) and Animal Ethics Committee (*REC 138–19*) of the Faculty of Veterinary Science, University of Pretoria, South Africa. Additionally, we received Sect. 20 approval from the Department of Agriculture, Forestry and Fisheries (DAFF) (Number: *12/11/1/1/8(1131)*).

Informed consent Written informed consent was obtained from owners and operators from cattle farms, cattle abattoirs, and retail outlets.

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