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Prevalence, Molecular Identification, Antimicrobial Resistance, and Disinfectant Susceptibility of *Listeria innocua* Isolated from Ready-to-Eat Foods Sold in Johannesburg, South Africa

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HIGHLIGHTS

- Prevalence of Listeria innocua was 21.3% (17 out of 80) in ready-to-eat food samples.
- Listeria detected in this study revealed 98-99% identity in 16S rRNA sequence with L. innocua.
- Most L. innocua isolates were susceptible to the studied commercial disinfectants.

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Acronyms and abbreviations

MIC=Minimum Inhibitory Concentration MRC=Manufacturer Recommended Concentration RTE=Ready-To-Eat

ABSTRACT

Background: Food contamination with *Listeria* spp. can occur at all stages of the food chain. The aim of this research was to investigate the prevalence, molecular identification, antimicrobial resistance, and disinfectant susceptibility of *Listeria innocua* isolated from Ready-To-Eat (RTE) foods sold in Johannesburg, South Africa.

Methods: Eighty RTE foods were collected from Johannesburg, South Africa. The 16S rRNA region of *L. innocua* isolates was amplified, sequenced, and identified using Basic Alignment Search Tool (BLAST). The antimicrobial resistance and disinfectant susceptibility (against four commercial disinfectants) of the isolates were evaluated using disk diffusion and microdilution assays. Data were statistically analyzed using SPSS v. 23.0.

Results: *Listeria* strains revealed a high 16S rRNA gene sequence analogy to *L. innocua* of between 98-99%. The overall prevalence of *L. innocua* was 21.3% (17 out of 80) in the RTE food samples. Most isolates were susceptible to the studied commercial disinfectants. All the *L. innocua* isolates from food sources were found to be resistant to ampicillin and cephalothin, while 83 and 74% of isolates were resistant to colistin sulphate and sulphatriad.

Conclusion: Prevalence of *L. innocua* was considerable in the RTE food samples sold in Johannesburg, South Africa. The *L. innocua* isolates showed high antibiotic resistance against ampicillin, cephalothin, colistin sulphate, and sulphatriad.

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Introduction

Listeria species are ubiquitous and can be found in food processing environments, soil, water, as well as in raw and processed food products (Ochiai et al., 2014; Sauders et al., 2012). Food contamination with *Listeria* spp. can occur at all stages of the food chain. *L. moncytogenes* and *L. innocua* are two species that are

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prevalent in food and food manufacturing environment. *L. monocytogenes* is the most dangerous pathogen and causes a disease known as listeriosis in humans (Gómez et al., 2014; Rodrigues et al., 2017). On the contrary, *L. innocua* is a non-pathogenic species and is often use as a *L. monocytogenes* surrogate in many laboratory experiment. The *Listeria* species can persist in food facilities for a few months to several years depending on the effectiveness of sanitization practices (Minarovičová et al., 2018). Various food types have often been found contaminated with *L. monocytogenes*, mostly foods of animal origin, raw vegetable salads, and Ready-To-Eat (RTE) foods. This raises potential for the people to consume food products contaminated with *Listeria* spp. (Lakicevic et al., 2015).

Some Listeria species such as L. innocua and L. monocytogenes which can contaminate foods have been found to form biofilms on food contact surfaces alongside other biofilm producing bacteria (Costa et al., 2018). Any persistence of L. innocua species with antimicrobial and disinfectant resistance on food contact surfaces can enhance survival and propagation of L. monocytogenes in the multi species biofilms (Hua et al., 2021). L. monocytogenes on resistant biofilm can crosscontaminate food in processing and retailing environment (Nyhan et al., 2020). Furthermore, the presence of resistant biofilm of L. innocua can negatively influence the efficacy of sanitisation processes, and hence the elimination of L. monocytogenes in food contact surfaces (Jung et al., 2017). The adaptation of L. innocua and its biofilm to disinfectant and sanitizers is mainly due to bacterial adaptation which can be attributed partly to the usage of incorrect concentrations during cleaning (Fox et al., 2015), and partly due to the acquisition of resistance genes from one bacterium (Mc Carlie et al., 2020).

Listeria species may acquire resistance against certain antibiotics, especially those that are used for treatment of listeriosis (Osaili et al., 2011). Antibiotic resistant *L. monocytogenes* can transfer resistance genes to *L. innocua* which can serve as reservoir for these genes (Escolar et al., 2017). Antimicrobial resistance has been associated with the over and under usage of antibiotics in mammals and aquaculture (Kumar and Pal, 2018). High antibiotic resistance of *L. monocytogenes* isolated from food has been reported for clindamycin, ampicillin, cephalothin, and sulphonamides (Carvalho et al., 2019). Due to its high prevalence in food and food contact surfaces, the contribution of *L. innocua* to the propagation and persistent of *L. monocytogenes* in food processing and retailing environment can be easily underestimated.

Hence, the aim of this research was to investigate the prevalence and molecular identification of *L. innocua* in RTE foods sold in Johannesburg, South Africa. Also,

antimicrobial resistance and disinfectant susceptibility of isolated *L. innocua* strains were evaluated.

Materials and methods

Sample collection

From February to November 2020, a total of 80 RTE foods were collected from outlets of Johannesburg, South Africa. Pasteurized milk (n=10), yoghurt (n=10), meat ham (n=10), and liver spreads (n=10) were bought from formal retail outlets, while beef stew (n=10), rice (n=10), tomato salad (n=10), and coleslaw salad (n=10) were bought from informal retail outlets. All samples in their original package were transported to the laboratory in a cold box (approx. 4 °C) and immediately analyzed. A total of four disinfectants commonly used in the food industry were kindly provided by a local manufacturer of detergents and disinfectants used in food and beverage industry. The disinfectants were classified into chlorinebased (BX), acid-based (PF and SC), and quaternary ammonium compound-based disinfectant (HG), based on their active ingredients.

Isolation and identification of Listeria species

The isolation and detection of *Listeria* species was conducted using the horizontal ISO method for the detection of *L. monocytogenes* and *Listeria* spp. (ISO, 2017). The identity of each *Listeria* spp. was confirmed by latex agglutination test using the OxoidTM Listeria Test Kit (Zymo Research, Irvine, USA), biochemical tests, and 16S rRNA sequencing. Pure cultures of bacterial isolates were stored in glycerol at -20 °C before identification and sequencing.

16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA extraction was carried out using Promega DNA purification kit (Promega Corporation, Madison, USA) following the manufacturer's instructions. The DNA quantity was ascertained with a NanodropTM 1,000, while the quantity was determined using agarose gel electrophoresis before further Polymerase Chain Reaction (PCR). The 16S rRNA region of each isolate was then amplified using universal primer pair of 27F (5'-AGAGTTTGATCMTGGCTCAG-3') 1492R (5'-CGGTTACCTTGTTACGACTT-3') (Dos Santos et al., 2019). For a of 25 µl reaction volume, the mixture consisted of 12.5 µl master mix, 1.25 μl of each primer (1 μM), 1 μl DNA (100 ng DNA) and 9 µl RNAse-free H2O (Thermo Fisher Scientific, Waltham, MA, USA). The amplification was done using the Mx3005P qPCR system (Agilent Technologies,

Waldbronn, Germany) using the following conditions: 5 min at 94 °C for initial denaturation, followed by 30 cycles of denaturation (1 min at 94 °C), annealing (90 s at 60 °C), and elongation (1 min at 72 °C). This was followed by a last final extension for 5 min at 72 °C (Soni and Dubey, 2014).

The amplified 16S rRNA region was purified by the Wizard® SV Gel and PCR Clean-Up System from Promega prior to sequencing. Sequencing was conducted using ABI 3500xl Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific) following the protocols outlined by Lane (1991) and Turner et al. (1999).

Prior to sequencing, the amplified DNA segments were edited using the Finch TV software version 1.4.0TM. Thereafter, DNA sequence alignment was done by Bioedit (software version 7.2.6.1 for Windows 10) and CLUSTAL W alignment tool was utilized as described by Usman et al. (2016). BLAST was done based on National Centre for Biotechnology (NCBI) website (http://blast.ncbi.nlm.nih.gov/). Evolutionary distance was done by the neighbor-joining tree under the Maximum Composite Likelihood method selection on Mega7 software (Kumar et al., 2016). Thereafter, a phylogenetic tree for the 17 isolates was developed using 16S rRNA nucleotide succession of *Listeria* spp. found in the GenBank.

Disinfectant susceptibility

The susceptibility of L. innocua to disinfectants was analyzed using triplicate microdilution and disk diffusion assays to ascertain reproducibility. For the microdilution method, disinfectants were diluted to the usage concentration recommended by individual manufacturer (BX: 0.1 mg/ml, PF: 20 mg/ml, HG: 30 mg/ml, and SC: 20 mg/ml). Fifty µl of Trypticase Soya Broth (Thermo Fisher Scientific, Waltham, MA, USA) was added to each well of 96-well plates. Thereafter, 50 µl of each disinfectant solution was placed in the first rows of the 96-well plates. Thereafter, two-fold serial dilution was conducted from the first row to the last one adding 50 µl of the mixture from the preceding row to the next one. The bacterial suspension was diluted to 0.5 McFarland and 100 µl of which was mixed with 10 ml of the diluent to obtain the final inoculum 5×10^5 Colony Forming Units (CFU)/ml. Fifty µl of the inoculum was dispensed in each well except the negative control wells. Ten µl PrestoBlue (Thermo Fisher Scientific, Waltham, MA, USA) was added into each well and incubated at 37 °C for 24 h. Minimum Inhibitory Concentration (MIC) values of disinfectant were determined as the first concentration in the wells at which no visible bacterial growth had occurred using visual observation (Tabit et al., 2016). The disk diffusion analysis was conducted using the Kirby-Bauer disk diffusion method (Hombach et al., 2013; McDonnell and Russell, 1999). Sterile Whatman filter paper disks of 6 mm in diameter were impregnated (20 μl) with each commercial disinfectant that had been diluted to the usage concentration recommended by individual manufacturers. The impregnated disks were then transferred aseptically onto Mueller Hinton Agar plates (Thermo Fisher Scientific, Waltham, MA, USA) which had been inoculated with 10 μl of each *L. innocua* culture (set at 1×10⁸ CFU/ml cell density). The inoculated plates were incubated at 37 °C for 24 h. Zone of inhibitions were measured by a ruler. Resistance to disinfectants were rated as either present (>5 mm) or absent (≤5 mm).

Antibiotic susceptibility

Antibiotic susceptibility of isolates was done in triplicate using Kirby-Bauer disk diffusion method on Mueller Hinton Agar (Thermo Fisher Scientific, Waltham, MA, USA). The antibiotic disks of ampicillin (10 μg), cephalothin (5 μg), colistin sulphate (25 μg), gentamicin (10 µg), streptomycin (10 µg), tetracycline (30 μg), sulphatriad (200 μg), and cotrimoxazole (25 μg) purchased from MAST Diagnostics (Merseyside, UK) were used. Each disk was aseptically placed on Mueller Hinton Agar plate which had been previously inoculated with 50 µl of bacterial suspension (previously set at 1×10^8 CFU/ml cell density). Up to four disks were placed at about 50 mm apart per plate and incubated at 37 °C for 24 h. The clear zone of inhibition of each antibiotic disk was measured and the level of susceptibility was determined using the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAT) breakpoints for Staphylococcus aureus considering that the breakpoints for Listeria species for the antibiotic disks used is not available (EUCAST, 2021).

Statistical analysis

Data analysis was conducted using SPSS version 23.0 in which MIC values were represented as mean±SD.

Results

Prevalence of L. innocua

The overall prevalence of *L. innocua* was 21.3% (17 out of 80) in the RTE food samples. Out of 10 samples per food group, *L. innocua* was orderly detected in pasteurized milk (4 cases), beef stew (3 cases), coleslaw (2 cases), yoghurt (2 cases), tomato salad (3 cases), liver spread (1 cases), ham (1 cases), and smoked Vienna (1 cases) making a total of 17 isolates. We did not find

L. innocua in rice samples. However, there was no significant difference (p>0.05) in the prevalence of *L. innocua* in the various RTE food groups.

Phylogenetic analysis of L. innocua strains

Nucleotide blast (BLASTn) of the amplified 16S rDNA sequences (Figure 1) of *Listeria* strains revealed 98-99% identity of 16S rRNA sequence with the one in *L. innocua*. Maximum composite likelihood revealed three different distinct phylogenetic clusters namely A, B, and C (Figure 2). The BLAST analysis showed that only one strain had low resemblance to the other *L. innocua* strains, but showed high resemblance to strains from other environments and geographical locations deposited in NCBI Genbank. *L. innocua* in clusters B and C from food sources appeared to be closely related but distant phylogenetically when compared to cluster A

Disinfectant susceptibility

Results from the micro dilution showed that only 2 out of the 17 isolates were found to be susceptible at MIC values greater than the Manufacturer Recommended Concentration (MRC) for usage in food processing facilities. An isolate from smoked Vienna had MIC values higher than the MRC for acid-based PF and SC. Similarly, one isolate from coleslaw had a value greater than the MRC for SC (Table 1). The result from the disk diffusion assay showed that most isolates were susceptible to HG, PF, and SC. Conversely, only three isolates were susceptible to BX (Table 2).

Antibiotic susceptibility

All the L innocua isolates from RTE food sources were

found to be resistant to ampicillin (10 μ g) and cephalothin (5 μ g), while 83 and 74% of isolates were resistant to colistin sulphate (25 μ g) and sulphatriad (200 μ g), respectively (Table 3). Conversely, 73, 69, and 60% of the isolates were susceptible to gentamicin (10 μ g), cotrimoxazole (25 μ g), and streptomycin (10 μ g) respectively, and 51% of the isolates were susceptible to tetracycline (30 μ g).

Discussion

In this study, L. innocua was isolated from 21.3% of RTE foods. Pasteurized milk, beef stew, and tomato salad showed the highest contamination. In a similar survey by Arslan and Özdemir (2020), L. innocua was found in 13.3% of RTE foods in Turkey. Also, Jamali et al. (2013) stated that L. innocua was the most common Listeria species isolated from raw milk in farm bulk tanks in Iran. with high prevalence rate of 57.8%. The presence of L. innocua isolates in RTE food samples could be due to post preparation contamination since Listeria spp. are heat sensitive (Alles et al., 2018). We revealed no significant difference in the prevalence of L. innocua in the various RTE food groups. This is probably due to this fact that contamination mostly occurs during/after processing by means of cross-contamination due to unhygienic food processing environment (Korsak and Szuplewska, 2016). Poor hygienic practices such as the holding food at incorrect temperatures favour the contamination and growth of L. innocua in foods (Von Holy and Makhoane, 2006). The major contributing factor in the cross-contamination of Listeria species is infective cleaning and sanitization in the food manufacturing and retailing environment (Costa et al., 2018).

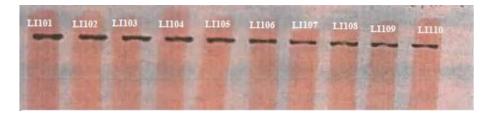


Figure 1: Agarose gel electrophoresis of amplified 16S rRNA gene of Listeria innocua isolates (LI101 to LI110)

Phylogenetic analysis was carried out to determine the genetic relatedness of *L. innocua* strains isolated from RTE foods in the current study. The results of phylogenetic analysis of *L. innocua* strains showed ongoing genetic diversification among isolates from RTE

food sources. The findings of the present research demonstrated the persistence and transient nature of *Listeria* species in the food processing and retailing environment as described previously in the study of Fagerlund et al. (2021).

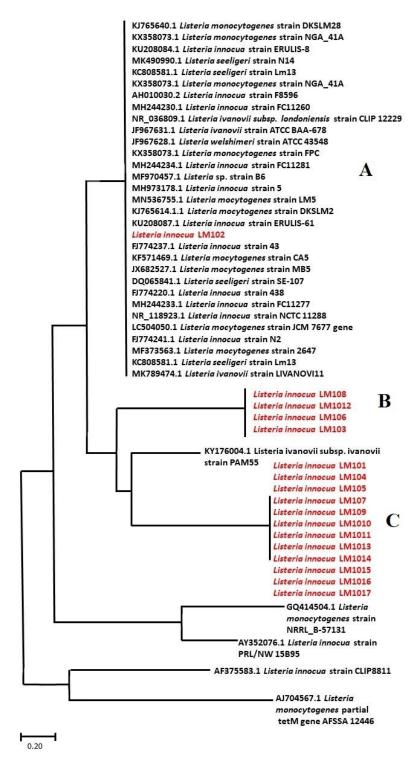


Figure 2: Phylogenetic analysis of 16S rRNA gene nucleotide sequences of *Listeria innocua* from ready-to-eat food by the neighbour-joining method, 1,000 bootstraps by the Tamura Nei model, comprising Polytomies A, B, and C with the *Listeria monocytogenes tetM* gene and *L. innocua* AF3755832 as outgroups.

Table 1: Minimum Inhibitory Concentrations (MIC) of disinfectants against Listeria innocua isolated from ready-to-eat foods in Johannesburg, South Africa

Strain code	Source	MIC (mg/mL)			
		BX^{CHL}	HG ^{QAC}	PF ^{AB}	SC ^{AB}
		0.1 MRC	20^{MRC}	30^{MRC}	20 ^{MRC}
LI 101	Tomato salad 1	0.10	30.00	5.00	5.00
LI 102	Smoked Vienna	0.05	30.00	20.00	40.00
LI 103	Coleslaw 1	0.05	20.00	20.00	40.00
LI 104	Liver Spread	0.05	5.00	5.00	20.00
LI 105	Yoghurt	0.01	10.00	10.00	10.00
LI 106	Yoghurt	0.05	2.50	2.50	2.50
LI 107	Ham	0.10	10.00	10.00	10.00
LI 108	Tomato salad 2	0.05	10.00	10.00	10.00
LI 109	Beef Stew 1	0.02	10.00	10.00	10.00
LI 110	Pasteurized milk 1	0.10	20.00	10.00	10.00
LI 111	Beef Stew 2	0.00	10.00	5.00	5.00
LI 112	Pasteurized milk 2	0.05	0.50	15.00	15.00
LI 113	Tomato salad 3	0.10	0.38	15.00	15.00
LI 114	Coleslaw 2	0.10	0.50	0.25	0.25
LI 115	Beef Stew 3	0.00	0.75	0.15	0.15
LI 116	Pasteurized milk 3	0.05	1.90	0.50	2.50
LI 117	Pasteurized milk 4	0.01	0.38	0.25	0.25

LI: Listeria innocua; CHL: Chlorine based disinfect; QAC: Quaternary Ammonium Compounds; AB: Acid based; NI: Not inhibited; MIC: Minimum Inhibitory Con-

centration; MRC: Manufacturer Recommended Concentration
Manufacturer Recommended Concentration (BX: 0.1 mg/ml; PF: 20 mg/ml; HG: 30 mg/ml; SC: 20 mg/ml)

All experiments were conducted in triplicate.

Table 2: Inhibition zone (mm) disinfectants against Listeria innocua strains from ready-to-eat foods in Johannesburg, South Africa

Strain code	Source	Inhibition zone (mm)			
		$\mathbf{B}\mathbf{X}^{\mathrm{CHL}}$	HG^{QAC}	PF ^{AB}	SC^{AB}
LI 101	Tomato salad 1	9.00±2.83	18.00±7.55	12.00±1.41	21.00±10.07
LI 102	Smoked Vienna	0.00	15.33±7.64	13.33±4.93	19.33±3.06
LI 103	Coleslaw 1	0.00	21.00±5.57	13.67±5.03	19.67±7.51
LI 104	Liver Spread	0.00	17.50±7.79	18.00±2.83	15.50±9.19
LI 105	Yoghurt 1	0.00	21.67±7.51	11.00±3.46	24.00±7.94
LI 106	Yoghurt 2	0.00	16.50±9.19	14.50±2.12	20.50±2.12
LI 107	Ham	11.00±7.07	13.33±3.21	13.33±3.21	16.00±2.65
LI 108	Tomato salad 2	0.00	10.00±6.08	9.67±3.21	16.50±17.68
LI 109	Beef Stew 1	0.00	14.50±10.61	9.50 ± 13.44	10.67±4.51
LI 110	Pasteurized milk 1	0.00	10.00±5.20	12.33±6.03	6.67±7.64
LI 111	Beef Stew 2	0.00	11.33±4.51	12.00±3.00	13.00±1.41
LI 112	Pasteurized milk 2	0.00	16.33±6.81	13.67±5.86	11.00±2.83
LI 113	Tomato salad 2	0.00	16.50±7.78	11.67±0.57	18.50±7.78
LI 114	Coleslaw 2	8.50±2.12	12.33±5.86	13.67±8.96	15.33±10.41
LI 115	Beef Stew 3	0.00	14.67±4.93	10.00±2.65	19.50±2.12
LI 116	Pasteurized milk 3	0.00	15.33±5.51	16.67±3.51	14.67±0.58
LI 117	Pasteurized milk 4	0.00	19.00±1.41	9.67±2.08	14.00±4.24

LI: Listeria innocua; CHL: Chlorine based disinfect; QAC: Quaternary Ammonium Compounds; AB: Acid based; MRC: Manufacturer Recommended Concentration

Manufacturer Recommended Concentration (BX: 0.1 mg/ml; PF: 20 mg/ml; HG: 30 mg/ml; SC: 20 mg/ml).

Resistance to disinfectants were rated as either present (>5 mm) or absent (≤5 mm) (McDonnell and Russell, 1999).

All experiments were conducted in triplicate.

Table 3: Antibiotic susceptibility of Listeria innocua strain from ready-to-eat foods in Johannesburg, South Africa

Antibiotic	Susceptibility frequency (%)		
	Resistant	Intermediate	Susceptible
Ampicillin (10 µg)	100	0	0
Cephalothin (5 µg)	100	0	0
Colistin Sulphate (25 µg)	83	0	17
Gentamicin (10 µg)	21	6	73
Streptomycin (10 µg)	40	0	60
Sulphatriad (200 µg)	74	6	20
Tetracycline (30 µg)	49	0	51
Cotrimoxazole (25 µg)	31	0	69

The susceptibility L. innocua was determined using the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for Staphylococcus aureus considering that the breakpoints for Listeria species for the antibiotic disks used is not available (EUCAST, 2021). All experiments were conducted in triplicate.

The MIC values of four commercially disinfectants that are commonly used in the food industry, namely BX (chlorine-based disinfectant), HG (Quaternary Ammonium Compounds disinfectant), and PF and SC (both acid-based disinfectant) were determined. Based on the microdilution assay, we found out that most of the L. innocua from RTE food sources were susceptible to all the four commercial disinfectants with MIC values lower or equal to the MRC of each disinfectant. L. innocua cells which have not developed some form of antimicrobial resistance are expected to be susceptible to these sanitizers under planktonic conditions, but this may not be the case in the presence of biofilms (Xu et al., 2019). Similarly, results from the disk diffusion assay showed that most isolates from food sources were susceptible to disks impregnated with HG, PF, and SC; but this was not in the case of BX. This discordance in the MIC values from the two assays was not expected as the findings from both assays were anticipated to be correlated. This disparity could be attributed to inefficiencies related to the diffusibility of the BX disinfectant in the disk (Benkova et al., 2020).

The resistance to disinfectants by Listeria species can occur due to the usage of sublethal concentrations and that cross-adaptation may occur for disinfectants with similar mode of inactivation (Lundén et al., 2003). It is worth noting that L. innocua can effectively be used as a surrogate of L. monocytogenes in the same food processing environment, especially in terms of disinfectant resistance (Costa et al., 2018). That is why the determination of the susceptibility of L. innocua to MRC is important, since it is of more practical interest to the food manufacturer than MIC values (Heir et al., 1995). L. innocua strains from RTE foods may acquire resistance and proceed to resist the MRC of commercial disinfectants. Therefore, effectiveness of disinfectants against microorganism must be continuously monitored in food facilities to ascertain the effectiveness of cleaning and disinfection of surfaces in food processing facilities and ensure production of safe food (Kastbjerg and Gram, 2012).

In this work, all the *L innocua* isolates from RTE food sources were found to be resistant to ampicillin and cephalothin. It was also found that 83 and 74% of isolates were resistant to colistin sulphate and sulphatriad, respectively. Similar to the findings of this study, Chin et al. (2018) indicted high levels of antibiotic resistance by *L. innocua* isolated from raw chicken and chicken related products in Malaysia. Antibiotic resistance is mainly associated with abuse in veterinary clinics and animal farms such as in the practice of adding antibiotics to animal feed as supplements (Roca et al., 2015). The high level of resistance displayed by many *L. innocua* isolates from our RTE food sources may be due to acquired

resistance emanating from the abusive usage of antibiotics (Shourav et al., 2020).

Antibiotic resistant genes in L. innocua may easily be transferred to other pathogenic bacteria such L. monocytogenes in the presence of an enabling environment (Adzitey et al., 2013). L. innocua from fruit holding facilities have been found to possess multi-drug resistant to more than three antibiotic (Jorgensen et al., 2021). A study on L. innocua isolates from RTE foods in Spain confirmed the presence of antibiotic resistant genes (Escolar et al., 2017). Gentamicin (10 µg) appeared to be the most effective antibiotic against L. innocua with 73% susceptibility and 6% intermediate susceptibility. Gentamicin susceptibility amongst L. monocytogenes isolates in humans has been widely reported; hence the antibiotic is regarded as a good treatment for listeriosis (Gómez et al., 2014; Walsh et al., 2001). It is worth noting that intermediate resistance of strains could possibly gain a fully-fledged resistance adaptation under certain favourable conditions. The presence of antibiotic resistant genes in pathogens can impede the successful treatment of individuals who have contracted food-borne disease with antibiotics a risk hence may lead to death (Ruiz-Bolivar et al., 2011).

Conclusion

The overall prevalence of *L. innocua* was considerable in the RTE food samples sold in Johannesburg, South Africa. We found no significant differences between prevalence rates of *L. innocua* and various RTE food groups. Most *L. innocua* isolates were susceptible to the studied commercial disinfectants which could be useful for cleaning and sanitization in food processing facilities. The *L. innocua* isolates showed high antibiotic resistance against ampicillin, cephalothin, colistin sulphate, and sulphatriad.

Author contributions

H.H.M., F.T.T., and B.C.D. designed the research, analyzed the data, and wrote the manuscript; H.H.M. conducted the experiment in capacity of a master's student. All authors read and revised the final manuscript.

Conflicts of interest

All the authors declared no conflict of interest.

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