Bacteriological investigation on *Listeria monocytogenes* in Egyptian food samples with special reference to its resistance patterns

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LIST OF ABBREVIATIONS

AK	Amikacin
ALOA	Agar *Listeria* according to Ottaviani and Agosti
AML	Amoxicillin
CAL	Ceftazidime + clavulanic acid
CDC	Centers for Disease Control and Prevention
CFU	Colony-forming unit
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CN	Gentamicin
Ct	Cycle threshold
DNA	Deoxyribonuceic acid
E	Erythromycin
EFSA	European Food Safety Authority
HACCP	Hazard analysis and critical control point
hlyA	Hemolysin gene
IAC	Internal Amplification Control
Iap	Invasion-associated protein
InlA	Internalin A
InlB	Internalin B
LIPI-1	*Listeria* pathogenicity island
LLO	Listeriolysin O
MPN	Most probable number
NCCLS	National committee for clinical and laboratory standards
NOR	Norfloxacin
PC-PLC	Phosphatidylcholine-specific phospholipase c
PCR	Polymerase chain reaction
PG	Penicillin G
PI-PLC	Phosphatidylinositol-specific phospholipase c
PrfA	Positive regulatory factor
RTE	Ready-to-eat food
SXT	Trimethoprim/ sulfamethoxazole
C30	Chloramphenicol

ABSTRACT

Listeria monocytogenes is among the most important foodborne pathogens. It may enter food processing environments through raw materials, handlers or equipment and may persist due to ineffective cleaning or sanitation. The bacterium can be isolated from both frozen vegetables and fresh food substances. This study aimed to estimate the prevalence of *L. monocytogenes* in spices and frozen vegetables and screen for some virulence factors and drug-resistance determinants of the isolated bacteria. First, conventional microbiological methods were used for the isolation and identification of bacteria. Next, the identity of isolated bacteria was confirmed by molecular techniques, and the virulence genes *iap* and *hlyA* were identified by real-time polymerase chain reaction (PCR). The hemolytic activity of the isolates was assessed by cultivation on sheep blood agar. Furthermore, the antimicrobial susceptibility of confirmed *L. monocytogenes* isolates was tested by the disk diffusion method against 10 antibiotics. Out of 331 vegetable samples, 47 isolates were confirmed to contain *L. monocytogenes*, whereas none of 40 spice samples tested positive. All isolates were positive for *iap* and *hlyA* genes. Susceptibility testing indicated that all isolates were sensitive to trimethoprim/sulfamethoxazole, but only 36% were sensitive to penicillin G, while 100% and 70% showed intermediate resistance to chloramphenicol and erythromycin, respectively. All tested isolates were resistant to amoxicillin, gentamicin and norfloxacin; on the other hand, 90, 86 and 84% of the tested strains were resistant to ciprofloxacin, ceftazidime/clavulanic acid and amikacin, respectively. In summary, *L. monocytogenes* isolates disseminated in frozen vegetable samples from the Egyptian market were highly virulent, entirely multiple-drug resistant and were enriched in iron-containing vegetables. Since *L. monocytogenes* is primarily pathogenic to humans and causes a life-threatening disease, there is a potential infection risk for people who usually deal with frozen vegetables before cooking. Hence, surveillance to *L. monocytogenes* in frozen products, together with implementation of tight measures would be valuable in preventing listeriosis, and are highly recommended.

Key words: *Listeria monocytogenes*- virulence genes- antibiotic resistance.