Occurrence and antibiogram of *Listeria monocytogenes* Isolates from Retail Meat Shops at Erbil City, Kurdistan Region, Iraq

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Abstract

Listeria monocytogenes is well-known globally as one of the most significant foodborne bacterial pathogens. Listeriosis may trigger life-threatening illness, such as severe sepsis, meningitis, sometimes resulting in lifelong harm and even death. This study aimed to determine the occurrence and antibiotic resistance pattern of *L. monocytogenes* in red meats sold at retail outlets in Erbil city, Kurdistan region, Iraq. Three hundred and seventy-five (375) samples were aseptically collected from retail meat shops between July and December 2018**.** For isolation of *L. monocytogenes*, samples were cultured on selective media and tested for their susceptibility to common antibiotics by disk diffusion assay. The results revealed that the overall occurrence of *L. monocytogenes* in red meat samples was 13.9%. Warm season was associated with increase in *L. monocytogenes* occurrence. The results of antimicrobial susceptibility testing showed that 98.1%, 94.2%, and 82.7% of isolates were resistant to Streptomycin, Gentamicin, and Ampicillin respectively. This resistance pattern of *L. monocytogenes* is critically alarming owing to the aforementioned antibiotics are the drugs of choice of treatment of listeriosis. This level of resistance requires further investigations and effective countermeasures since it may pose a public health hazard.

Introduction

Listeria monocytogenes is a Gram-positive, motile, non-spore-forming, facultative anaerobic short rods (0.2 × 0.5 to 2 µm) widely distributed in nature and is frequently isolated from a variety of sources, including soil, mud, decaying vegetation, contaminated silage, fecal materials (Buchanan *et al*., 2017). *L. monocytogenes* can thrive and grow in a cold, moist environment and also survives in the intestinal tract of at least 37 species of mammals, both domestic and wild. It has the capability to grow over a wide range of temperature ranging from 1°C to 45°C, also it survives in harsh conditions, such as a wide range of pH range (4.4 and 9.4), in high salinity (40% w/v), low water activity (aw) (≥0.92), and hypoxic conditions (Thigeel, 2010; Singh *et al*., 2019).

L. monocytogenes is an intracellular pathogen causing listeriosis in human and other mammals, a disease with significant public health risk. Indeed, listeriosis is one of the greatest hazardous foodborne zoonoses, life-threatening disease responsible for high mortality rate reaching 20-30%. Almost all individuals are susceptible to infection but at-risk populations are pregnant women, neonates, young children, weak or aging people, and those who are immunocompromised. Other conditions that may increase susceptibility to listeriosis are diabetes, cirrhosis, asthma, and ulcerative colitis. The incubation period ranges from three days to several weeks (3 to 70 day), after ingestion of a contaminated item, with a medium of 3 weeks. Listeriosis does not spread from person-to-person except from infected mother to child in utero or at birth (Murray *et al*., 2018; Das, 2019).

Listeriosis can cause a diversity of symptoms, depending on the individual and the part of the body affected. Generally, the infection is characterized by flu-like symptoms including fever, myalgia, and sometimes, gastrointestinal symptoms. If infection expansion to the nervous system, symptoms may development to include severe headache, confusion, stiff neck, loss of balance or convulsions (Murray *et al*., 2018; Davis *et al*., 2019). Not only the epidemiology of human and animal listeriosis are still largely unknown, but also the data regarding antimicrobial uses and susceptibility pattern of *L. monocytogenes* isolates are extremely scarce in Iraq.

L. monocytogenes can be found in some handled foods like processed meats and dairy products owing to post-processing contamination. The food of animal origin such as different types of red meat, poultry meat, dairy products, fish, and seafood are excellent vehicle for transmission and listerial growth, therefore, it can easily be contaminated if it is wrongly handled or stored even in a refrigerator (Lambertz *et al*., 2012; Desai *et al*., 2019). Contamination of meat and meat products by *L. monocytogenes* is reported from various developed and developing countries (Maktabi *et al*., 2015; Almashhadany *et al*., 2016; Salim *et al*., 2017; Arsalan and Baytur, 2018; Olanya *et al*., 2019). Red meat is an important vehicle for *Listeria* transmission in 99% of listeriosis cases (Kurpas *et al*., 2018; Amusan and Sanni, 2019). Since the isolation of the first multidrug-resistant *L. monocytogenes* strain at France in 1988, several strains isolated from food, clinical, and environmental samples have shown resistance to one or more antibiotics. The levels of resistance vary among strains and are also influenced by antimicrobials use in humans and animals and geographical differences (Noll *et al*., 2018; Matle *et al*., 2019). To date, less is known about the occurrence of *L. monocytogenes* in raw red meat and their antibiogram at Erbil city, Kurdistan region. Therefore, the goals of this work were to determine the occurrence and antibiotic susceptibility profile of *L. monocytogenes* in red meats sold at retail outlets in Erbil city.

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Materials and Methods

Study design and sampling

A total of 375 raw meat samples from cattle, sheep, and goats (125 of each) were randomly and aseptically collected from retail shops in different places of Erbil city during the period from July to December 2018 according to previously published method (Almashhadany, 2019). Collected samples were placed in separate sterile polyethylene bags within cold container and transported to Pathological Analysis Department, Knowledge University.

Isolation and identification of *L. monocytogenes*

Under sterile laboratory conditions, samples were cut into small sections by sterile blades to release bacteria into the enrichment broth. A total of 25 gm of red meat (as the optimal sample size) were suspended in 225 ml of *Listeria* Enrichment Broth (LEB) (HiMedia, India) and incubated for 48 hours at 37°C. After incubation period, 0.1ml of the LEB was streaked onto *Listeria* Oxford Medium Base (OXA) plate (HiMedia ,India) and incubated at 37°C for 48 h in microaerophilic atmosphere (Alsheikh *et al*., 2013). After incubation period, suspected colonies were examined for typical *L. monocytogenes* morphology including iridescence alongside with Gram stain findings. The biochemical characterization included (Oxidase, Catalase, Indole, H_2S production, Urease, Methyl red Voges-Proskauer, haemolysin production, hippurate hydrolysis, and citrate utilization tests) (Almashhadany *et al*., 2016).

Production of virulence-related enzymes

All isolated were tested for the production of virulence-related enzymes; lipase, protease, and DNase according to standardized procedures (Leber *et al*., 2016).

Antibiotics susceptibility testing

Modified Kirby-Bauer disk diffusion assay on Mueller-Hinton agar containing 0.5% defibrinated sheep blood was employed to test for sensitivity of *L. monocytogenes* strains to a panel of generally used antibiotics according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2011). After 24 h of incubation, the zones of inhibition were measured (mm), and the isolates were categorized as susceptible, intermediate, or resistant to specific antibiotics according to CLSI published breakpoints. The tested antibiotics were: chloramphenicol, tetracycline, kanamycin, gentamicin rifampicin, ampicillin, ciprofloxacin, vancomycin, streptomycin, and trimethoprim.

Statistical analysis

Data were analyzed using SPSS software version 21. Confidence intervals are exact Clopper-Pearson confidence intervals. Chi-Square test was employed to test the difference between samples.

Results

Occurrence of *L. monocytogenes*

Out of 375 red meat samples, 13.9% (52) were positive for the presence of *L. monocytogenes*. Of note, 16.8% of beef samples harbored *L. monocytogenes*. The distribution of positive samples is summarized in Table1. Statistically, it is estimated that up to 17.78% (95% confidence interval) of red meat in Erbil retail outlets are contaminated by *L. monocytogenes*. Despite the apparent difference in occurrence between samples of beef and mutton, the difference is not statistically significant (χ^2=1.621, P=0.2029).

Temporal distribution and pathogenicity of *L. monocytogenes*

The highest occurrence *of L. monocytogenes* was found in August (22.6%) (Figure 1) and the lowest occurrence was in December (81%). It is apparent that the decrease in *L. monocytogenes* occurrence in meat is correlated with the progress of the second half of the year with a good correlation coefficient (r^2=0.70). Regarding the ability of *L. monocytogenes* to produce virulence-related enzymes, catalase, haemolysin production, lipase, protease, and DNase, were detected (Table 2). Generally, it was found that all the 52 isolates of *L. monocytogenes* were 100% positive for catalase & haemolysin production, while 88.5% of isolates produce DNA hydrolytic enzyme(s).

Antibiotics susceptibility pattern of *L. monocytogenes*

L. monocytogenes isolates (n=52) were tested against 10 antibiotics. The results of antibiotic susceptibility testing are showed in Figure 2. Strikingly, high resistance was found to the drugs of choice for treatment of serious listeriosis; gentamicin (94%) and ampicillin (82%). Moreover, great proportion of isolates showed intermediate susceptibility to tetracycline (80.8%) and rifampicin (84.6%).

Discussion

Infections of *L. monocytogenes* are associated with high fatality rate of approximately 30% worldwide and hospitalization

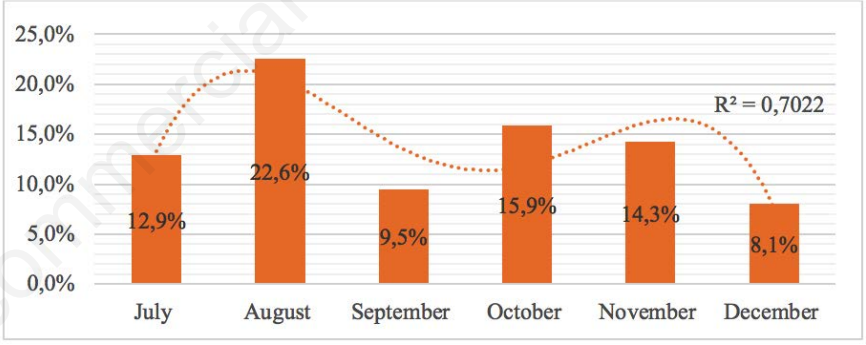

Figure 1. Temporal distribution of *L. monocytogenes* in red meat samples.

Table 1. Prevalence of *L. monocytogenes* among red meat.

Type of meats	No. examined	Positive samples n (%)	95% CI
Beef	125	21 (16.8)	10.71-24.53
Mutton	125	14 (11.2)	6.26-18.08
Goats	125	17 (13.6)	8.13-20.88
Total	375	52 (13.9)	10.53-17.78

Table 2. Ability of *L. monocytogenes* isolates to produce virulence-related enzymes.

Enzyme	Positive isolates. No. %	95% CI
Catalase	52 (100)	93.15-100
Haemolysin production	52 (100)	93.15-100
Lipase	48 (92.3)	81.46-97.86
Protease	41 (78.8)	65.30-88.94
DNase	46 (88.5)	76.56-95.65

rate of more than 95%. This fatality rate is the highest recorded among foodborne pathogens, thus making *L. monocytogenes* one of the most dangerous foodborne pathogens globally (Leong *et al*.,2016; Buchanan *et al*., 2017; Matle *et al*., 2019). In this study, the occurrence of *L. monocytogenes* in red meat samples was 13.9% (Table 1). Such occurrence reflects inadequate hygienic practices in the preparation of red meat at the retail level. These results are consistent with a large South African study which found the occurrence of *L. monocytogenes* in meat and meat products samples was 14.7% (Matle *et al*., 2019). However, lower rates were reported in previous study in Erbil city 3.6% (Alzubaidy *et al*., 2013), India (5.3%) (Sran *et al*., 2015), Turkey (8.5%) (Sanlıbaba *et al*., 2018), and Nigeria (7.4%) (Amusan and Sanni, 2019).

In contrast, higher rates were reported from Iran (28.05%) (Mashak, 2015), Yemen (22.9%) (Almashhadany *et al*., 2016), Erbil city (28.1%) (Salim *et al*., 2017), and Turkey (26.6%) (Arslan and Baytur, 2018). These varying occurrence rates may be attributed to difference in geographical location, hygienic condition of meat shops and workers associated with slaughtering and handling meat at different levels, the amount and source of samples collected, laboratory detection methods, and seasonal variations (Singh *et al*., 2019).

The higher percentage of contaminated red meat samples were found in beef 21/125 (16.8%). This may be a direct result of exposure to many potential contaminating sources due to its wide popularity and preference by consumers in Erbil city. It is important to pay attention to the source of contamination of raw red meats. This type of meat needs different stages of processing at the slaughterhouse including slaughtering, skinning, evisceration and other steps to produce the final meat sold to consumers. Meat and meat products are stored under refrigeration and the absence of competitive bacteria along with suitable a_w and pH conditions allow *L. monocytogenes* to multiply successfully (Meloni, 2015).

In terms of temporal distribution of *L. monocytogenes*, the highest occurrence was in August and late autumn (Figure 1). Several studies had linked warm season to high occurrence of *L. monocytogenes*. Rhoades and associates reported that the isolation of *L. monocytogenes* was higher during warmer months which were compatible with our result in present study (Rhoades *et al*., 2009). Furthermore, a Turkish study documented that the isolation rates were found to be the highest in autumn, while the rates were low in spring (Elmali *et al*., 2015). Additionally, a Greek study reported that *L. monocytogenes* in the summer was higher than in winter, spring and rainy seasons (Effimia, 2015). Further supporting reports were emerged from Finland (Sjoman, 2010) and Iran (Fallah *et al*., 2012). On the other hand, contradictory observations also reported (Guerini *et al*., 2007; Fallah *et al*., 2013; Modzelewska-Kapituła *et al*., 2014). It seems that strains of *L. monocytogenes* have different temporal variation preference (Fallah *et al*., 2012; Wang *et al*., 2015).

In the present work, the result of In-vitro pathogenicity tests illustrated that all the 52 isolates of *L. monocytogenes* were (100%) positive for catalase, haemolysin production, and Hippurate Hydrolysis, while (92.3%), (78.8%) and (88.5%) produce Lipase, Protease, and DNase consecutively. This study identified all 52 isolates of *L. monocytogenes* to be positive for haemolysin production and hippurate hydrolysis. Thus, have role in pathogenicity of the bacterium (Alzubaidy *et al*., 2013).

Antibiogram profile of *L. monocytogenes* isolates is illustrated graphically in Figure 2. *L. monocytogenes* is naturally susceptible to penicillins; the drug of choice for human listeriosis alone or combined with an aminoglycoside (Miller *et al*., 2014, Sanlibaba *et al*., 2018). In the present study, (82.7%) *L. monocytogenes* isolates showed resistance to the β-lactam antibiotics, which is highly significant from clinical and public health perspectives. Resistance to β-lactam antibiotics are scarce in *L. monocytogenes* strains. No β-lactamase enzyme has been found in clinical or environmental isolates. However, anrB efflux pump is responsible for decreased susceptibility to various β-lactams including ampicillin in *L. monocytogenes* (Collins *et al*., 2010). In a recent study conducted by Matle and associates in 2019 in South Africa, all the tested isolates showed resistance to at least 3 of the 19 antibiotics (Matle *et al*., 2019). Resistance to streptomycin (99.0%), clindamycin (97.3%), fusidic acids (95.6%), nitrofurantoin (79.7%), and gentamycin (74.4%) was observed. However, Wu and colleagues found that penicillin G was the only antibiotic to which all *L. monocytogenes* isolates were susceptible (Wu *et al*., 2015). Resistance to streptomycin, tetracycline, chloramphenicol, and erythromycin was found in *L. monocytogenes* to be under control of transferable plasmid (Poyart-Salmeron *et al*., 1990). Indeed, aminoglycosides (*i.e.* streptomycin and gentamicin) are deactivated by bacterial aminoglycoside-modifying enzymes whose genes may be chromosomally or extra chromosomally encoded (Ramirez & Tolmasky, 2010). It has been reported that streptomycin resistance was attributed to 6-aminoglycoside nucleotidyltransferase or other listerial gene(s) homologous to *cat221* gene (encoding a chloramphenicol acetyltransferase) in *Streptococcus* and *Enterococcus* (Poyart-Salmeron *et al*., 1990; Hadorn *et al*., 1993; Morvan *et al*., 2010).

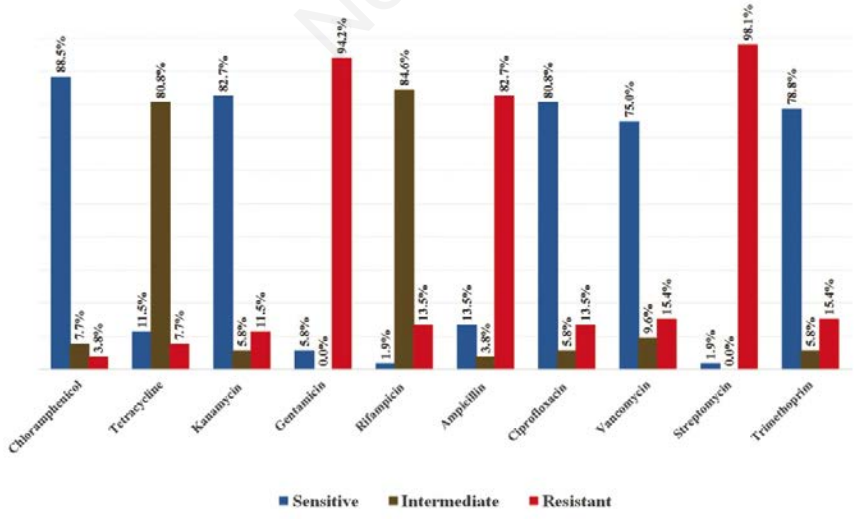

Figure 2. Antibiotic sensitivity of *L. monocytogenes* isolates.

Conclusions

In conclusion, this study has expanded existing knowledge by illustrating the occurrence of *L. monocytogenes* in retail red meat in Erbil City. Epidemiological and molecular investigations should follow this and the previous studies to assess the risk for consumers. Random antibiotics usage should be restricted to minimize public health hazards of spreading multi-drug

resistance pathogens. A four-season study is recommended for further investigation on the distribution of *L. monocytogenes* isolates in various meat samples accompanied by antimicrobial susceptibility testing. Obeying the rules of good hygiene practices (GHP), Good Manufacturing Practices (GMP), and Hazard Analysis & Critical Control Point (HACCP) in the slaughterhouses and manufacturing process can significantly decrease the occurrence of *L. monocytogenes*.

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