ISSN 1996-3351

Asian Journal of Biological Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2019.671.676

Research Article
Incidence and Antimicrobial Susceptibility of *Listeria monocytogenes* Isolated from Different Food Sources in Enugu, Nigeria

Eneh Chiamaka, Nweze Emeka and Eke Ifeanyichukwu

Department of Microbiology, University of Nigeria, Nsukka, Nigeria

Abstract

Background and Objective: *Listeria monocytogenes* is a facultative intracellular parasite which has been isolated from different food samples and has been associated with different food-borne epidemics. Though the organism was initially recognized as an animal pathogen, consumption of foods heavily contaminated with the pathogen leads to listeriosis, a disease which might be of epidemic proportions in humans. This infection is one of the highest causes of morbidity among food-borne pathogens and may have a fatality rate as high as 20-30%. Despite its public health importance, there are not much information available on the incidence of *Listeria* spp. in food samples sold in south-eastern Nigeria.. This study was thus performed to fill this knowledge gap. **Materials and Methods:** A total of 240 food samples which included vegetables (garden eggs, cucumbers and carrots) and raw meat (beef, goat and pork) were randomly collected from different local markets and commercial outlets in Enugu state, Nigeria. Isolation and identification of *Listeria* spp. were performed using standardized microbiological protocols. Molecular analysis was performed in order to detect the presence of common virulence genes such as *hly* and *iap* from the organism. Antimicrobial susceptibility screening of the recovered *L. monocytogenes* isolates was also carried out to ascertain their resistance pattern to a panel of antibiotics. **Results:** *Listeria* spp. (n = 75) were isolated from 240 food samples (which include vegetables and meats) collected from different commercial outlets in Enugu, Nigeria. *Listeria monocytogenes* was the most isolated species (40%) and had varying incidence in cucumber (20%), garden egg (17.5%), carrot (12.5%), goat meat (10%), beef (7.5%) and pork (7.5%). Antimicrobial susceptibility screening of the *L. monocytogenes* isolates showed the organism to exhibit high resistance against ceftazidime (96.67%), cloxacillin (90%), cefuroxime (86.67%), augmentin (86.67%), ceftriaxone (80%) and erythromycin (66.67%). On the other hand, most of the isolates were susceptible to ofloxacin (76.67%) and gentamicin (60%). Also, molecular studies were carried out to determine the presence of haemolysin gene (*hly*) and invasion-associated protein (*iap*) 12 *Listeria monocytogenes* isolates. Surprisingly, none of the targeted genes were found in the isolates considered. This, we opined, may be due to a number of factors which include but are not limited to a spontaneous mutation or a subtle mismatch during amplification. **Conclusion:** The high incidence of antibiotic-resistant *L. monocytogenes* isolates in the area under consideration is quite alarming and this calls for more stringent food regulatory procedures to curtail the spread of the pathogen.

Key words: Nigeria, *Listeria*, antimicrobial susceptibility, amplification

Citation: Eneh Chiamaka, Nweze Emeka and Eke Ifeanyichukwu, 2019. Incidence and antimicrobial susceptibility of listeria monocytogenes isolated from different food sources in Enugu, Nigeria. Asian J. Biol. Sci., 12: 671-676.

Corresponding Author: Nweze Emeka Innocent, Department of Microbiology, University of Nigeria, Nsukka, Nigeria Tel: +234 8068535841

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Listeria monocytogenes is a Gram-positive, rod-shaped, motile and non-sporulating bacteria which have been isolated from diverse environmental sources such as milk, sewage, vegetables, fruits, foods, water, soils, animals and humans[1,2]. It is a ubiquitous organism whose unique physiology adds to its defiance to adverse environmental stresses. For instance, it is psychrophilic and can grow very well under a wide temperature range[3] of -0.4-50°C. It withstands high salt concentrations or osmotic pressure; grows under a broad pH range and survives under aerobic to microaerophilic conditions[4-7]. Furthermore, proteins such as internalins-surface-active molecules that promote the binding and phagocytosis of the pathogen into epithelial cells; pore-forming listeriolysin O which allows the engulfed listeria to escape into the cytoplasm of epithelial cells; iron-binding siderophores, zinc-dependent protease and phosphatidylinositol-specific phospholipase mediate the pathogenicity of the organism[6-9]. These virulent factors enable the pathogen to exhibit a life cycle of intracellular parasitism and escape from the "ever-preying eyes" of the innate and adaptive immune system[4,8,10].

Though *L. monocytogenes* was initially recognized as an animal pathogen, consumption of foods heavily contaminated with the pathogen leads to listeriosis, a disease which might be of epidemic proportions in humans[3]. This infection is one of the highest causes of morbidity among the food-borne pathogens and may have a fatality rate as high[3,6,9,11] as 20-30%. In addition to typical symptoms associated with gastrointestinal infections, listeriosis may also affect the central nervous system and circulation systems[10,12] manifesting as meningitis, encephalitis, mother-to-fetus infection and septicaemia[8]. While healthy individuals may be affected, immunosuppressed individuals such as pregnant women, infants, aged people and AIDS patients are the major population groups at risk to invasive listeriosis[1,3,10,13,14].

In spite of the high mortality rate and the increasing antibiotics resistance profile of *L. monocytogenes*[11], there are few studies documenting the incidence of the pathogen in Nigeria. This paucity of research and publicity hampers the development of public health policies and regulations on the control of the pathogen. The aims of this study were therefore: (1) To determine the occurrence of *Listeria* spp. and *L. monocytogenes* in fruits and vegetables sold in Enugu, south-eastern Nigeria, (2) Establish the antibiotics susceptibility patterns of the isolated *L. monocytogenes* and finally, (3) To evaluate the presence of invasion-associated protein (*iap*) gene and listeriolysin O gene (*hly*) in the isolated species.

MATERIALS AND METHODS

Sample collection: A total of 240 food samples which included vegetables (garden eggs, cucumbers and carrots) and raw meat (beef, goat and pork) were randomly collected from different local markets and commercial outlets in Enugu state, Nigeria. The samples were properly labelled and immediately transported to the Department of Microbiology Laboratory, University of Nigeria, Nsukka for analysis.

Isolation and identification: The ISO 11290 method adapted with little modifications[1] was used in the isolation and identification of *Listeria* spp. Briefly described, the food samples were first homogenized using a sterile laboratory mortar and pestle. Exactly 1 g of the homogenized sample was added into 9 mL of half Fraser broth. This served as the first or pre-enrichment medium. After a 24 h-incubation at 37°C, 0.1 mL of the incubated half Fraser broth was added to 10 mL Fraser (second enrichment medium) and was incubated at 37°C for 48 h. This was followed by the inoculation of a loopful of the enriched Fraser broth into PALCAM selective agar and incubating for 24 h at 37°C. Colonies that show a black centre or halo typical of *Listeria* spp. were selected and re-streaked on tryptic soya media supplemented with 5% yeast extract. All the presumptively-identified *Listeria* isolates were further subjected to standard biochemical tests such as Gram reaction, oxidase test, catalase test, motility test, haemolysis test and fermentation of sugars such as glucose, xylose, rhamnose, mannose and mannitol.

Antibiotics susceptibility test: The Clinical and Laboratory Standards Institute (CLSI)[15] reference procedure M100-S22 was used for the disk diffusion susceptibility test. Exactly 30 *L. monocytogenes* isolates were subjected to sensitivity discs (Abtek, Liverpool, United Kingdom) containing a variety of antibiotics such as ceftazidime (30 μg L^{-1}), cefuroxime (30 μg L^{-1}), gentamicin (10 μg L^{-1}), ceftriaxone (30 μg L^{-1}), erythromycin (5 μg L^{-1}), cloxacillin (5 μg L^{-1}), ofloxacin (5 μg L^{-1}) and augmentin (30 μg L^{-1}). After incubation, zones of inhibition around the discs were accordingly interpreted as "susceptible," "intermediate" or "resistant" using the CLSI break points. *Staphylococcus aureus* ATCC25923 was used as the reference strain.

Molecular detection of virulence genes using polymerase chain reaction (PCR): About12 *L. monocytogenes* isolates were subjected to molecular analysis in order to detect the presence of common virulence genes such as *hly* and *iap* genes. The isolates were first grown in tryptic soy broth for 72 h and harvested by centrifuging for 5 min. Using a DNA extraction kit (Zymo Research, Irvine, USA), the DNA of the cell pellets was extracted as per the manufacturer's specifications. For PCR, oligonucleotide primer pairs of 5'-GCAGTTGCAAGCGCTTGGAGTGAA-3' and 5'-GCA ACG TAT CCT CCA GAG TGA TCG-3' specific for *hly* (Seifi, 2012) and 5'-ACA AGC TGC ACC TGT TGC AG-3' and 5'-TGA CAG CGT GTG TAG TAG CA-3' specific for *iap*[16] were used. The reaction mixture (25 μL) was composed of 100 ng of DNA template, 1 μM of the forward and backward primers each, 2.5 μL of 10X PCR buffer, 0.2 mM dNTP mix, 2 mM $MgCl_2$ and 1 unit of *Taq* DNA polymerase. The PCR cycling conditions include an initial denaturation of DNA at 94°C for 2 min, followed by 30 cycles each consisting of a 30 sec-denaturation step at 95°C, an annealing time of 30 sec at 55°C and an extension period of 30 sec at 72°C. This was followed by a final extension at 72°C for 10 min, with the resultant PCR products being held at 4°C. The amplified products were analyzed using agarose gel electrophoresis (1%), stained with ethidium bromide and observed under an ultraviolet transilluminator.

RESULTS AND DISCUSSION

Determining the incidence and prevalence of *Listeria monocytogenes* is important in establishing the epidemiological profile of listeriosis within an area. Nigeria, the most populated black nation on earth has been hampered by the issue of poor health infrastructures, inaccessibility to clean water, loosened food hygiene regulations and proximity of local markets to unclean environments. All of these factors help in increasing the risk of listeriosis. Unfortunately, there are few research works documenting the incidence and prevalence of *L. monocytogenes* in the country.

In this study, a total of 240 food samples collected from Enugu state, south-eastern Nigeria were considered for the incidence of *Listeria* spp. As shown in Table 1, *Listeria* spp. were mostly found in cucumber (55%), followed by goat and beef samples with an incidence of 32.5% each, carrot (25%), pork samples (22.5%) and garden eggs (20.0%). Furthermore, out of the 75 isolates which were gotten from the food samples, most (40%) were *L. monocytogenes* while the remaining proportion was unequally shared by other listeria species. On speciation (Table 2), *L. monocytogenes* had the highest occurrence in cucumber (20%), garden egg (17.5%) and carrot (12.5%), while the highest occurring species in beef samples was *L. grayi* (12.5%). In goat meat, *L. welshimeri* carried the lead (12.5%) and was closely followed by *L. monocytogenes* (10%) while *L. monocytogenes* and *L. ivanovii* had equal occurrence in pork samples. The isolation of *Listeria* spp. from food samples may be due to the use of dung slurries or faeces of infected animals or carriers as manures for improved crop production[5]. The use of unprocessed water for irrigation may also be responsible for the incidence of listeria in food samples[12]. It is also important to highlight that this foodborne pathogen has also been isolated from fish meat, raw chicken, raw milk, egg and cheese[17-19].

With the rise in antibiotics resistance and dearth of novel antibiotics discovery, there is a global call for increased surveillance of pathogenic strains for antibiotics resistance. Previously-susceptible strains may acquire antibiotics resistance genes through conjugation, transduction or transformation. The isolated *L. monocytogenes* strains were subjected to antibiotics susceptibility screening. As shown in

Table 1: Incidence of *Listeria* spp. in raw meat samples and vegetables

Samples	No. of examined sample	No. of positive sample (%)
Beef	40	13 (32.5)
Goat meat	40	13 (32.5)
Pork	40	9 (22.5)
Cucumber	40	22 (55.0)
Carrot	40	10 (25.0)
Garden egg	40	8 (20.0)
Total	240	75 (100.0)

Table 2: Occurrence of different species of *Listeria* in raw meat and vegetable samples

	No. of isolated species (%)					
Samples	*L. monocytogenes*	*L. ivanovii*	*L. grayi*	*L. innocua*	*L. welshimeri*	*L. seeligeri*
Beef	3 (7.5)	4 (10.0)	5 (12.5)	1 (2.5)	0.0	0.0
Goat meat	4 (10.0)	3 (7.5)	0.0	1 (2.5)	5 (12.5)	0.0
Pork	3 (7.5)	3 (7.5)	1 (2.5)	1 (2.5)	0.0	1 (2.5)
Garden egg	7 (17.5)	1 (2.5)	0.0	0.0	0.0	0.0
Cucumber	8 (20.0)	5 (12.5)	1 (2.5)	2 (5.0)	5 (12.5)	1 (2.5)
Carrot	5 (12.5)	3 (7.5)	1 (2.5)	0.0	1 (2.5)	0.0
Total No. (%)	30 (40.0)	19 (25.3)	8 (10.7)	5 (6.7)	11 (14.7)	2 (2.7)

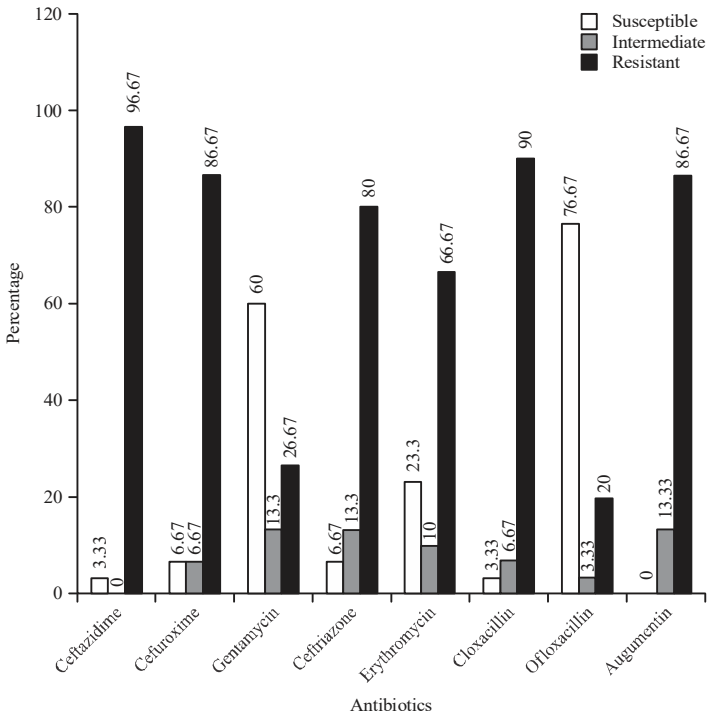

Fig. 1: Antibiotics susceptibility patterns of *L. monocytogenes*

Fig. 1, most of the isolates were resistant to ceftazidime (96.67%), cloxacillin (90%), cefuroxime (86.67%), augmentin (86.67%), ceftriaxone (80%) and erythromycin (66.67%) while ofloxacin and gentamicin inhibited 76.67 and 60% of the tested isolates, respectively. High resistance of *L. monocytogenes* to other antibiotics such as ampicillin (92%), rifampicin (84%), rifamycin (84%) and florfenicol (66%) has been documented in literature[11]. Other studies have also shown the multi-drug resistance profile of *L. monocytogenes*[3,5,12]. While this is in line with the general increasing trend of antibiotic resistance among bacteria groups[3], we hypothesized that such alarming resistance of *L. monocytogenes* to a wide variety of antibiotics may be due to the environmental exposure of the organism which enables it to acquire adaptive features such as antibiotic-resistance genes from other organisms. Other authors found that there was a high incidence of L. monocytogenes contamination in raw beef, pork and chicken sold in Makurdi with the recovered isolates showing resistance to erythromycin, gentamicin, cotrimoxazole and chloramphenicol but resistant to augmentin, amoxicillin, tetracycline and cloxacillin[20].

Fingers can also be pointed to the indiscriminate use of antibiotics in the treatment of animal diseases and as growth promoters in animal production[3,9,11]. In contrast to the European Union which have restricted the use of antibiotics in animal production, most countries in Africa such as Nigeria are still experiencing unchecked administration of antibiotics in animal production. This problem is worsened by inadequate health regulations on the certification of meat products prior to consumption.

In order to curtail the spread of *L. monocytogenes*, a look into the molecular determinants of its pathogenicity is expedient. Undoubtedly, the most characterised virulence gene of the pathogen is the haemolysin gene, *hly* (formerly called *hly*A or *lis*A). This gene codes for listeriolysin O, a pore-forming cytolysin which enables the pathogen to escape from the phagosome of the host cell. Other virulent genes in *L. monocytogenes* include the *plc*A which codes for a phosphatidylinositol-specific phospholipase; *mpl*, which codes for a metalloprotease; *act*A, required for the polymerization of actin filaments during cell-to-cell spread of the pathogen; *iap* gene, responsible for the invasiveness of the pathogen; *inl*A and *inl*B, coding for internalins and *prf*A, the virulence regulator[21,22]. In this present study, none of the tested isolates showed any presence of *iap* or *hly* virulent genes. This surprising result may be due to a subtle mismatch between the primers and the targeted DNA. Furthermore, the absence of these virulent determinants may be due to the occurrence of a spontaneous mutation or deletion of the

genes, leading to their alteration[22,23]. A slightly similar result was observed by Ndahi *et al.*[24] when they reported the presence of *hly* gene in only one out of 12 *L. monocytogenes* isolates considered in their study. They opined that such low occurrence of the haemolysin gene among the considered isolates may be due to their the non-virulent nature when present in the environment. On the other hand, Cao *et al.*[21] showed the presence of virulence genes which include but were not limited to *hly* and *iap* in nine isolates of *L. monocytogenes* considered in their study.

CONCLUSION

The high incidence of antibiotic-resistant *L. monocytogenes* isolates in the area under consideration is quite alarming and this calls for more stringent food regulatory procedures should be used to curtail the spread of this pathogen. Of interest too is the isolation of other *Listeria* species from food samples sold in the southeastern Nigeria. A further investigation of their virulence genes of is warranted to understand their pathogenicity.

REFERENCES

1. Jamali, H., L.C. Chai and K.L. Thong, 2013. Detection and isolation of *Listeria* spp. and *Listeria monocytogenes* in ready-to-eat foods with various selective culture media. Food Control, 32: 19-24.
2. Lakicevic, B., I. Nastasijevic and M. Raseta, 2015. Sources of *Listeria monocytogenes* contamination in retail establishments. Proc. Food Sci., 5: 160-163.
3. Walsh, D., G. Duffy, J.J. Sheridan, I.S. Blair and D.A. McDowell, 2001. Antibiotic resistance among *Listeria*, including *Listeria monocytogenes*, in retail foods. J. Applied Microbiol., 90: 517-522.
4. Low, J.C. and W. Donachie, 1997. A review of *Listeria monocytogenes* and listeriosis. Vet. J., 153: 9-29.
5. David, O.M. and A.T. Odeyemi, 2007. Antibiotic resistant pattern of environmental isolates of *Listeria monocytogenes* from Ado-Ekiti, Nigeria. Afr. J. Biotechnol., 6: 2135-2139.
6. Radoshevich, L. and P. Cossart, 2018. *Listeria monocytogenes*: Towards a complete picture of its physiology and pathogenesis. Nat. Rev. Microbiol., 16: 32-46.
7. Abdollahzadeh, E., S.M. Ojagh, H. Hosseini, E.A. Ghaemi, G. Irajian and M.N. Heidarlo, 2016. Antimicrobial resistance of *Listeria monocytogenes* isolated from seafood and humans in Iran. Microbial Pathogenesis, 100: 70-74.
8. Hamon, M, H. Bierne and P. Cossart, 2004. *Listeria monocytogenes*: A multifaceted model. Nat. Rev. Microbiol., 4: 423-434.
9. Morobe, I.C., C.I. Obi, M.A. Nyila, M.I. Matsheka and B.A. Gashe, 2012. Molecular Characterization and Serotyping of *Listeria monocytogenes* with a Focus on Food Safety and Disease Prevention. In: Biochemical Testing, Jimenez-Lopez, J.C. (Ed.). InTech Inc., Rijeka, Croatia, ISBN: 978-953-51-0249-6, pp: 197-206.
10. Drevets, D.A. and M.S. Bronze, 2008. *Listeria monocytogenes*: Epidemiology, human disease and mechanisms of brain invasion. FEMS Immunol. Med. Microbiol., 53: 151-165.
11. Srinivasan, V., H.M. Nam, L.T. Nguyen, B. Tamilselvam, S.E. Murinda and S.P. Oliver, 2005. Prevalence of antimicrobial resistance genes in *Listeria monocytogenes* isolated from dairy farms. Foodborne Path. Dis., 2: 201-211.
12. Nwachukwu, N.C., F.A. Orji, I. Iheukwumere and U.G. Ekeleme, 2010. Antibiotic resistant environmental isolates of *Listeria monocytogenes* from anthropogenic lakes in Lokpa-Ukwu, Abia State of Nigeria. Aust. J. Basic Applied Sci., 4: 1571-1576.
13. Seifi, S., 2012. Prevalence and risk factors for *Listeria monocytogenes* contamination in Iranian broiler flocks. Acta Scient. Vet., 40: 1-6.
14. Lotfollahi, L., A. Chaharbalesh, M.A. Rezaee and A. Hasani, 2017. Prevalence, antimicrobial susceptibility and multiplex PCR-serotyping of *Listeria monocytogenes* isolated from humans, foods and livestock in Iran. Microb. Pathogenesis, 107: 425-429.
15. CLSI., 2012. Performance standards for antimicrobial susceptibility testing: Twenty-second informational supplement. CLSI Document M100-S22, Vol. 32, No. 3, Clinical and Laboratory Standards Institute, Wayne, PA., USA., January 2012.
16. Indrawattana, N., T. Nidabbhasobon, N. Sookrung, M. Chongsa-Nguan, A. Tungtrongchitr, S. Makino and W. Chaicumpa, 2011. Prevalence of *Listeria monocytogenes* in raw meats marketed in Bangkok and characterization of the isolates by phenotypic and molecular methods. J. Health Popul. Nutr., 29: 26-38.
17. Khan, J.A., R.S. Rathore, S. Khan and I. Ahmad, 2013. *In vitro* detection of pathogenic *Listeria monocytogenes* from food sources by conventional, molecular and cell culture method. Braz. J. Microbiol., 44: 751-758.
18. Salihu, M.D., A.U. Junaidu, S.B. Manga, M.L. Gulumbe and A.A. Magaji *et al.*, 2008. Occurrence of *Listeria monocytogenes* in smoked fish in Sokoto, Nigeria. Afr. J. Biotechnol., 7: 3082-3084.
19. Yakubu, Y., M.D. Salihu, O.O. Faleke, M.B. Abubakar and A.U. Junaidu, *et al.*, 2012. Prevalence and antibiotic susceptibility of Listeria monocytogenes in raw milk from cattle herds within Sokoto Metropolis, Nigeria. Sokoto J. Vet. Sci., 10: 13-17.
20. Portnoy, D.A., T. Chakraborty, W. Goebel and P. Cossart, 1992. Molecular determinants of *Listeria monocytogenes* pathogenesis. Infect. Immun., 60: 1263-1267.

21. Cao, X., Y. Wang, Y. Wang and C. Ye, 2018. Isolation and characterization of *Listeria monocytogenes* from the black-headed gull feces in Kunming, China. J. Infect. Public Health, 11: 59-63.
22. Cooray, K.J., T. Nishibori, H. Xiong, T. Matsuyama, M. Fujita and M. Mitsuyama, 1994. Detection of multiple virulence-associated genes of *Listeria monocytogenes* by PCR in artificially contaminated milk samples. Applied Environ. Microbiol., 6: 3023-3026.
23. Rawool, D.B., S.V.S. Malik, S.B. Barbuddhe, I. Shakuntala and R. Aurora, 2007. A multiplex PCR for detection of virulence associated genes in *Listeria monocytogenes*. Int. J. Food Saf., 9: 56-62.
24. Ndahi, M.D., J.K.P. Kwaga, M. Bello, J. Kabir, V.J. Umoh, S.E. Yakubu and A.J. Nok, 2014. Prevalence and antimicrobial susceptibility of *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus* strains from raw meat and meat products in Zaria, Nigeria. Lett. Applied Microbiol., 58: 262-269.