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Occurrence and Antibiotic Susceptibility of *Listeria* Species Isolated from Ready-to-eat Mixed Vegetable Salad Sold in Fast Food Eateries in Port Harcourt

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Aims: The study aim to evaluate the occurrence and antibiotic susceptibility of *Listeria* species in ready-to-eat (RTE) mixed vegetable salad sold in selected fast food eateries in Port Harcourt.
Study Design: This work was based on a completely randomized design with two replications and the average values calculated for mean comparison.
Place and Duration of Study: The study was done in Food and Industrial Microbiology Laboratory, Department of Microbiology, University of Port Harcourt, Nigeria, between June and December, 2018.
Methodology: The study evaluated the occurrence and antibiotic susceptibility of *Listeria* species isolated from 100 samples of ready-to-eat (RTE) mixed vegetable salad sold in selected fast food eateries in Port Harcourt, using standard procedures involving Fraser broth, polymycin? acriflavin lithium chloride ceftazidime aesculin mannitol (PALCAM) agar, Mueller Hinton agar and multiple antibiotic disc.
Results: Of the 100 samples examined, 14% were positive for *Listeria* species. The *Listeria* isolates were identified on the basis of their physiological and biochemical characteristics as *L. innocua* (56.52%), *L. welshimeri* (34.78%) *L. grayi* (8.70%). The antibiotic susceptibility results reveal varying resistance against gentamicin (8.69%), augmentin (66.67%), amoxicillin (58.33%), erythromycin (25.00%), tetracycline (45.83%), cotrimoxazole (37.50%), chloramphenicol (41.67%) and cloxacillin (62.50%).

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Conclusion: Although no *L. monocytogenes* was detected in the samples examined, the presence of *L. innocua* often reported as masking *L. monocytogenes* in growth media is a source of concern to public health experts and a potential threat to consumers.

Keywords: Antibiotic resistance; eatery; Listeria; public health; vegetable salad.

1. INTRODUCTION

Ready-to-eat (RTE) mixed vegetable salad which is now a major part of our daily meal in Nigeria and sold in fast food eateries is a mixture of fresh vegetables (carrot, cabbage, cucumber, green pepper, and lettuce), pre-packed baked beans and cream milk. The consumption of vegetable salad in Nigeria and other parts of the world has greatly increased based on the requirements of nutrients, vitamin A, iron, calcium, proteins, fats, minerals and the medical benefits of its constituents [1,2,3,4,5,6].

Although the consumption of fresh and minimally processed vegetables is considered healthy, outbreaks related to the contamination of these products are frequently reported since they are not subjected to treatments that considerably reduce microbiological hazards [7]. Vegetables can be contaminated from different environmental sources, such as soil, water, insects, air, birds, animal or equipment during cultivation, harvesting, processing, transport and marketing [6,8,9]. A large number of pathogenic microorganisms including *Listeria monocytogenes, Salmonella*, *Escherichia coli* O157:H7, *Bacillus anthracis*, *Mycobacterium* spp., *Brucella* spp., *Yersinia enterolytica*, *Clostridium perfringens*, *Klebsiella* spp. and *Mycobacterium para-tuberculosis* have been reported to be associated with contamination of vegetables [6]. Generally vegetables have 10^3 to 10^5 microorganisms/cm^3 or 10^4 to 10^7 microorganisms/g [6].

Listeria monocytogenes is a ubiquitous bacterium that can be found in faeces, sewage, untreated irrigation water or surface water, dust, soil and fertilizer used on farm sand in decaying plant matter, making the presence of this bacterium in vegetables a continual risk [10,11,12,13,14].

Listeria monocytogenes is naturally susceptible to a range of antibiotics that act on Gram-positive bacteria [15]. With the bacterium having a remarkable ability to develop resistance to every antibiotic, it can be anticipated that even bacterial species such as *Listeria*, which were considered to be susceptible to ampicillin, aminoglycosides, tetracycline, macolides, vancomycin, carbenicillin, cephaloridine, chloramphenicol, erythromycin, furazolidone, mvillin, neomycin, novobiocin, oleandomycin, ticarcillin, azlocillin and less susceptible to chlortetracycline, oxytetracycline, tetracycline, gentamycin, kanamycin, nitrofurantoin, penicillin G, streptomycin, will evolve towards multi-resistance [16,17,18]. According to Gómez et al. [19], most strains of *L. monocytogenes* show natural resistance to current fluo-roquinolones and cephalosporins, and especially those of the third and fourth generations, such as cefotaxime and cefepime, and to fosfomycin, oxacillin and lincosamides.

This study therefore, aim to evaluate the occurrence and antibiotic susceptibility of *Listeria* species isolated from ready-to-eat mixed vegetable salad in selected fast food eateries in Port Harcourt, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 100 ready-to-eat mixed vegetable salad samples without dressing were purchased from 10 fast food eateries in Port Harcourt metropolis and immediately transferred to the Microbiology Laboratory, University of Port Harcourt. The *Listeria monocytogenes* PCM 2191 serovar 01/2 which served as positive control was obtained from the Polish Collection of Microorganisms, Poland.

2.2 Isolation of *Listeria* Species

The techniques recommended by the United States Department of Agriculture (USDA) [20] and the Health Products and Food Methods of the Government of Canada [21] were employed using Fraser broth (Oxoid, England) and Polymixin acriflavin lithium chloride ceftazidime aesculin mannitol agar (PALCAM) (Oxoid, England).

2.2.1 Primary selective enrichment

Twenty-five grams of each sample was transferred to a stomacher bag containing 225 ml of sterile half-strength Fraser broth. The mixture was homogenized using a stomacher (Lab-Blender, Seward Medical, London) at high speed (state speed) for 1-2 min. The test portion was incubated at 30°C for 24 h.

2.2.2 Secondary selective enrichment

Full strength Fraser broth with full concentration of supplement was employed. From the pre-enrichment culture (half-strength Fraser broth), 0.1 ml was transferred into 10 ml of full-strength Fraser broth and was incubated at 35°C for 24-48 h.

2.2.3 Isolation

From the culture obtained in Fraser broth showing evidence of darkening due to aesculin hydrolysis by *Listeria* spp., 0.1 ml was transferred onto duplicate PALCAM plates. After spreading, plates were incubated at 37°C for 24-48 h. The plates were examined for the presence of characteristic colonies presumed to be *Listeria* sp 1- 2 mm greyish-green colonies with a black sunken centre and a black halo on a cherry-red background, following aesculin hydrolysis and mannitol fermentation. Typical colonies were selected randomly from a pair of PALCAM plates for confirmation and subsequent identification.

2.3 Biochemical Test

Colonies suspected to be *Listeria* were transferred onto trypticase soy agar (Becton, Dickinson & Company, France) with 0.6% yeast extract (LAB M, UK) and incubated at 37°C for 18 to 24 h, before being subjected to the following standard biochemical tests: Gram staining, catalase reaction, oxidase reaction, beta haemolysis on sheep blood agar and carbohydrate fermentation using mannitol, rhamnose and xylose as described by Janzten et al. [22] and Jemmi and Stephan [23].

2.4 Antibiotic Sensitivity

Antibiotic resistance was assessed by the disk diffusion assay according to CLSI guidelines using the breakpoints of *Staphylococcus* species resistance since no resistance criteria exist for *Listeria* susceptibility testing in the CLSI guidelines [24,25,26]. Antibiotics disk containing the following antibiotics: Tetracycline (30 µg), amoxicillin (20 µg), augmentin (20 µg), gentamycin (10 µg), erythromycin (15 µg), cloxacillin (5 µg), chloramphenicol (30 µg) and cotrimoxazole (20 µg) were employed (Abtek biological, UK). From an overnight culture in brain heart infusion broth, a 10^8 cell/ml (0.5 MacFarland turbidity standards) bacterial culture was prepared in sterile saline, from which 0.1 ml was inoculated onto Mueller Hinton agar. Thereafter, antibiotic discs were aseptically placed on the surface of the agar and plates incubated at 37°C for 24 h. Zone of inhibition was measured in millimeter (using what instrument).

3. RESULTS AND DISCUSSION

The microbiological quality of partially processed vegetables as found in pre-packed mixed vegetable salads should be of great concern since freshly-cut vegetables are reported to be vehicle for the transmission of food-borne pathogens.

In this study, *Listeria* species were targeted and detected in mixed ready-to-eat (RTE) vegetable salad samples purchased from 5 of the 10 fast food eateries with the percentage occurrence ranging from 6.67 to 25.00% (Table 1). Of the 100 samples analyzed, *Listeria* species were isolated from fourteen (14%) samples. The presence of *Listeria* species in these RTE vegetable salads is a pointer to the fact that the vegetables were not poorly processed since a number of authors have reported the presence of *Listeria* in vegetables and mixed pre-packed vegetable salad globally [10,14,27,28,29,30,31].

The *Listeria* species were identified on the basis of the morphological and biochemical characteristics as *L. innocua* 13 (56.52%), *L. welshimeri* 8 (34.78%) and *L. gray* 2 (8.70%) (Table 2). No *Listeria monocytogenes* was detected in the samples examined. Ajayeoba et al. [30] have reported low incidence of *Listeria monocytogenes* in RTE vegetable in traditional markets in South-Western Nigeria while Lin et al. [32] detected *L. monocytogenes* in 1 of 63 vegetable salads served in 31 food service facilities in Brazil. The inability to detect *L. monocytogenes* may not also be unconnected with the presence of raw carrot, which has been reported as bactericidal to *L. monocytogenes* [33], in the homogenate overnight in the primary enrichment broth coupled with the high percentage of *L. innocua* known to produce inhibitory bacteriocin against *L. monocytogenes* in growth media [34].

Table 1. Percentage occurrence of *Listeria* species from the selected fast food eateries

Eateries code	No. of samples examined	No. of positive samples	% occurrence
DSAR	12	3	25.00
DSCB	7	0	0.00
SMCB	20	4	20.00
GNNR	14	3	21.43
GNWL	5	0	0.00
GNUT	6	1	6.67
EMUP	17	3	21.43
GNRL	6	0	0.00
PTRK	5	0	0.00
DSRK	8	0	0.00
Total	100	14	

Table 2. Physiological and biochemical characteristics of *Listeria* species

Listeria	Number	Gram reaction/shape	Catalase	Oxidase	Nitrite reduced to nitrate	Haemolysis	Xylose	Rhamnose	Mannitol
Listeria monocytogenes PCM 2191 serovar 01/2	1	+ve/rod	+	-	-	+	-	+	-
L. grayi	2	+ve/rod	+	-	+	-	-	-	+
L. welshimeri	8	+ve/rod	+	-	-	-	+	V	-
L. innocua	13	+ve/rod	+	-	-	-	-	-	+

The results of the antibiotic sensitivity (Table 3) showed a high resistance to augmentin (66.67%) followed by cloxacillin (62.50%) and amoxicillin (58.33%). The least resistance was obtained for gentamycin (8.33%). The antimicrobial resistance profile reveals that majority of the *Listeria* spp. were resistant to at least an antibiotic. The multiple-antibiotic resistance observed in the non-pathogenic strains poses a threat of transferring same to other bacteria or *L. monocytogenes*. A number of authors have reported varying levels of resistance of *Listeria* spp. and *L. monocytogenes* from different sources to tetracycline, gentamycin, chloramphenicol, augumentin, cotrimoxazole and erythromycin [35,36,37,38,39,40,41,42,43]. Specifically, the susceptibility of *Listeria* spp. from salad vegetables and vegetable salad sold in Zaria, Nigeria to chloramphenicol (42.86%) and erythromycin (64.29%) are comparable to the results of this study [44]; but not in agreement with the 71.43% and 75.00% reported by Ieren et al. [44] and Bryne et al. [14] against tetracycline respectively. The susceptibility of *Listeria* spp. isolated from vegetable items sold by local and super shops in

Table 3. Distribution of the antibiotic susceptibility of the 23 isolated *Listeria* and *Listeria monocytogenes* PCM 2191 serovar 01/2 to common antibiotics

Antibiotic	Resistance	Intermediate	Sensitive
Amoxicillin	14 (58.33%)	4 (16.67%)	6 (25.00%)
Augmentin	16 (66.67%)	3 (12.50%)	5 (20.83%)
Chloramphenicol	10 (41.67%)	5 (20.83%)	9 (37.50%)
Cloxacillin	15 (62.50%)	3 (12.50%)	6 (25.00%)
Co-trimoxazole	9 (37.50%)	8 (33.33%)	7 (29.17%)
Erythromycin	6 (25.00%)	3 (12.50%)	15 (62.50%)
Gentamycin	2 (8.33%)	3 (12.50%)	19 (79.17%)
Tetracycline	11 (45.83%)	8 (33.33%)	5 (20.83%)

Dhaka city to gentamycin (75.00%) is also comparable to the findings of this study and consistent with the high and often 100% sensitivity of *Listeria* isolates to gentamycin [39,43,45]. The range of antibiotics to which resistance was observed in the study is of a great concern since antibiotics such as erythromycin, tetracycline and chloramphenicol were previously effective in the treatment of listeriosis [16].

4. CONCLUSION

This study has demonstrated the presence of *Listeria* species in ready-to-eat mixed vegetable salad from selected fast food eateries in Port Harcourt. Though *L. monocytogenes,* the potential human pathogen was not detected; the high presence of *L. innocua* is a cause for concern since it produces a bacteriocin against *L. monocytogenes* in growth media. An increased antibiotic resistant was demonstrated in line with worldwide pattern indicating a potential public health risk. It seems an impossible task protecting food samples from such defilement?, but with proper handling and processing, the challenge can be practically controlled to a very large extent.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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