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Isolation and Characterization of *Listeria monocytogenes* from Different Indigenous Vegetables Consumed in Okigwe, Imo State – Nigeria

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Abstract: The objectives of this study were to evaluate the presence of *Listeria monocytogenes* in three vegetables consumed as indicated in Nigeria and investigate the antibiogram of the isolates. *Telferia occidentalis, Solanum macrocarpon*, and *Pterocarpus soyauxii* were purchased, and blended in the microbiology laboratory, Abia State University, Uturu – Nigeria. The homogenate was cultivated into Listeria Selective Agar, after enrichment at 37°C for 24 – 48 hours. Out of a total sample size of 40, *listeria monocytogenes* had frequency of occurrence of 60%, 25% and 60% from *Telferia occidentalis, Pterocarpus soyauxii, Solanum macrocarpon* respectively. The mean *Listeria monocytogenes* load of the vegetable samples ranged between 0.8 x 10^4cfu/g – 4.38 x 10^4cfu/g. Multidrug resistance against aminoglyoxides, β-lactam, rifampicin was also documented. Cephalosporins such as Avicef, Ciprofsin including fluoroquinolones such as siprosan, and drovid showed significant bactericidal action against the *Listeria monocytogenes* isolates. These observations are of public health significance. Government of the World should intensify campaign against food borne diseases such as listeriosis with their research communities.

Key words: Food borne diseases, listeriosis, Nigerian Vegetables, Environmental Health.

INTRODUCTION

Food borne diseases are among the most serious health problems affecting the public, development in all parts of the world. (Prescott *et al.,* 2005). Industrialization, mass fast food production, and human migration have disseminated and increased the incidence and severity of food borne diseases worldwide (Chukwu *et al.,* 2006)

Listeriosis is among the most common bacterial foodborne pathogens worldwide and the disease is emerging as an important food borne pathogen of public health concern (Chukwu *et al.,* 2006).

Listeriosis is caused by a gram positive, rod, and facultative anaerobic bacterium called *Listeria monocytogenes.* The organism is motile by means of peritrichous flagella at 20 – 25°C, and non-motile at 37°C. (Arora, 2004; Cheesbrough, 2006).

Listeria monocytogenes is widely distributed in nature and can be found on decaying vegetation, soils, animal faeces, sewage, and water.

Animal products such as milk, diary products also get contaminated and passes infection to man (Andurier and Martin, 1989; Beuchat and Brackett, 1996).

The organism is acid tolerant, psychrotolerant, salt tolerant, and this might throw explanation on the reason for the contamination of lactic acid fermentation products even at refrigeration temperature (Madigan *et al.,* 2000).

Worthy of note is the fact that infections of *Listeria monocytogenes* are rare, but when it occurs, it most frequently affect pregnant women in their last trimester, new born, children, adults whose immunity is compromised by diseases such as cancer or acquired immune deficiency syndrome (Aureli *et al.,* 2000).

Outbreaks of foodborne listeriosis in Halifax, Nova Scotia, California through contaminated cabbage have been reported. Talaro, (2005), Frazier and Westhoff, (2004).

Reports of listeria infection in ground water, pasteurized milk, raw milk, under cooked sea food, cabbage have also been documented Talaro, (2005).

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Clinical features of listeriosis ranges from mild influenza – like symptoms to meningitis, still birth and abortion in pregnant women (Gowendolyn and Engelkirk, 2000). Other symptoms reported include: gastroenteritis, persistent fever and granulomas (Whitelock-Jones *et al.,* 2001; Alfonso *et al.,* 2004).

In relatively recent time, reports of emergence of antibiotic resistant *Listeria monocytogenes* have remained of significant public health concern. Multi-drug resistance to erythromycin, tetracycline, dicloxacillin, trimethroprim – sulfamethoxazole has been reported (Rodaz-Saurez *et al.,* 2006; Brooks *et al.,* 2004).

The aims and objectives of this study are to evaluate the presence of *Listeria monocytogenes* in three vegetables (*Pterocarpus soyauxii, Telferia occidentalis, and Solanum macrocarpon*) which are commonly consumed in Nigeria, and to investigate the antimicrobial susceptibility patterns of the isolated *Listeria monocytogenes.*

MATERIALS AND METHODS

The test vegetable samples; *Telfairia occidentalis, Solanum macrocarpon, and Pterocarpus soyauxii* were purchased from different markets, following random sampling in Okigwe, Imo State of Nigeria. Forty samples of each of the vegetables were studied.

The vegetables were transported with sterile cellophane papers to the Microbiology Laboratory, Faculty of Biological and Physical Sciences, Abia State University, PMB 2000 Uturu - Nigeria. Equipments and materials used in the study included ohaus digital weighing balance, KAE electronic incubator, zeiss electronic compound microscope, American pressure sterilizer (Autoclave). Hockey Stick Spreader, Homogenizer, Listeria Selective Agar (Oxoid Ltd, Basing Stoke, England, CM0856) Blood Agar (Antech), Nutrient Broth (Antech), Antibiotic Sensitivity Disc (Optun Laboratories, Aba – Nigeria), Gram's Reagents, Sugars: Glucose (BDH), Sucrose (BDH), Rhamnose (M & B), Xylose (BDH), Maltose (M & B) and Kovac's Reagent.
The entire reagents used were of analyte grade.

Media Preparation:

All microbiological media used in this study were prepared according to the manufacturers instructions and autoclaved at 121°C, 15psi for 15 minutes. The ready to use semi solid agar was aseptically dispensed into disposable petridishes, and allowed to cool and gel.

Isolation of Listeria Monocytogenes from the Vegetable Samples:

Listeria monocytogenes are psychrophilic, and the vegetables were kept in the refrigerators for 24 hours before culture (Cowan, 1973).

The test vegetables were ground into a pulp using a sterile homogenizer. The resulting pulps (homogenate) were used for the study.

1 gram of each homogenate was first dispensed in a test tube containing 9mls of sterile nutrient broth. This was incubated at 37°C within 18hrs to revive viable but non-culturable cells of *listeria*.

1ml of nutrient broth suspension after 18 hours of incubation, was taken with sterile pipette onto Listeria Selective Agar. The selective agar was incubated in a microaerophilic condition for 24 – 48 hours.

Bioload of the vegetable was determmed using spread plate technique and glass hockey stick spreader (Cowan, 1973). The isolates were subjected to characterization to identify *Listeria monocytogenes* from *Listeria ivanovii*, *L. innocua*, *L. welshieri*, *L. seeligeri*, *L. grayi*, *L. murayi*, and *L. denitrificans*.

The procedures for the characterization of isolates were previously reported by Arora, (2004), Cheesbrough, (2006), James (2005).

Gram positive rods, motile at 25°C – 30°C, which were Catalase + ve, Oxidase –ve, Indole –ve, CAMP +ve, Glucose, sucrose + rhamnose, and maltose fermentation +ve, xylose fermentation –ve were referred to as *Listeria monocytogenes.* Arora, (2004).

RESULTS AND DISCUSSION

Out of 40 samples of each test vegetables, *Listeria monocytogenes* was isolated from *Telferia occidentalis. Pterocarpus soyauxii, Solanum macrocarpon* in 60%, 25%, and 60% prevalence rates respectively (Fg. 1). The mean listeria loads of the vegetables were 1.8 x 10^4cfu/ml, 4.38 x 10^4cfu/ml, 0.8 x 10^4cfu/ml for *Telferia occidentalis, Solanum macrocarpon, and Pterocarpus* samples respectively (Table 1)

Listeria monocytogenes strains isolated from *Telferia occidentalis* showed multi-drug resistance against Aminoglycosides such as lincocin, streptomycin, chloramphenicol, erythromycin, β – lactan antibiotics such as ampiclox, floxapen. (Table 2).

However, avicef, siprosan, drovid, and ciprofsin showed high bactericidal action against *Listeria monocytogenes* isolated from *Telferia occidentalis* (Table 2).

Listeria monocytogenes isolates from *solanum macrocarpon* were 29.62%, 25%, 22% susceptible to Fluoroquinolones such as ciprofloxacin, norfloxacin and drovid.

Similarly, isolates of *Listeria monocytogenes* from *Pterocarpus soyauxii* were 12.50%, 16.60%, susceptible to ciprofloxacin, norfloxacin respectively. (Table 1).

The isolates were 50% susceptible to rifampicin. The isolates were also 50% resistant to amipiclox, a first generation β-lactam antibiotic (Table 1). Isolates from *Pterocarpus soyauxii* remained 75% resistant to floxapen, lincocin. (Table 1).

Antimicrobial Susceptibility Testing of Isolates of Listeria monocytogenes from Different Vegetables studied / Analysed:

Antibiotic susceptibility test was carried out on all the isolates of listeria monocytogenes using paper disc diffusion technique. A 0.5ml of 12 hour peptone water culture was used to inoculate on a dry sterile nutrient agar. This was spread over the entire surface of the nutrient agar using a sterile glass spreader and allowed to dry at about 15 – 30 minutes. The antibiotic disc was placed far from each other to avoid their zones of inhibition from coalescing into the other. The plates with the antibiotic discs were incubated at 37°C for 24 hours to observe the zones of growth inhibition produced by the antibiotics.

Standards of the National Clinical Committee on Clinical Laboratory Standards (NCCLS) were used to determine if isolate is resistant or susceptible to a given antibiotic.

These procedures used for antimicrobial susceptibility testing were previously reported by Chigbu and Ezeronye, (2003)

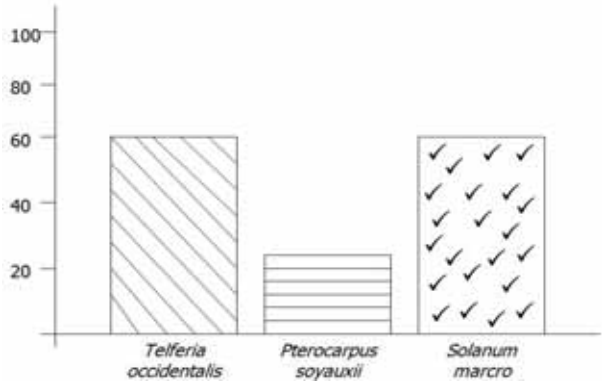

Fig. 1: Percentage Occurrence of *Listeria monocytogenes* in Vegetables.

Table 1: *Listeria Monocytogenes* Load in Samples of *Telferia occidentalis, Solanum macrocarpon, and Pterocarpus soyauxii*

Vegetable sample	Sample Size	Mean Colony Forming Units (cfu/g)
Telferia occidentalis	40	1.8×10^4
Pterocarpus soyauxii	40	4.38×10^4
Solanum marcrocarpon	40	0.8×10^4

Sample size = 40 for all the vegetable studied.

Discussion:

A total of 40 vegetable samples were screened for the presence of *Listeria monocytogenes*. *Telferia occidentalis, and Solanum macrocarpon* had 60% frequency of occurrence while *Pterocarpus soyauxii* had 40% frequency of occurrence. The 60% frequency is relatively high especially in *Telferia occidentalis,* which is a very popular fresh leafy vegetable with a wide consumption rate.

Findings from the results show that *Listeria monocytogenes* occurs in the vegetable samples to varying bioloads. *Solanum macrocarpon* and *Telferia occidentalis* had mean colony forming units of 4.38×10^4cfu/g and 1.8×10^4cfu/g respectively. The higher prevalence of the organism in both samples could perhaps be attributed to the fact that both vegetables are closer to ground, and are usually in contact with farmland, soil manure etc.

Pterocarpus soyauxii had very low frequency of occurrence. This is because the vegetable is the leaf of a tall tree. It is not commonly in contact with the soil, unless during harvesting, handling. Generally, the presence of the pathogen in the test vegetables raises enough concern due to its recorded high pathogenicity.

Table 2: Antibiotic susceptibility Patterns *of Listeria monocytogenes* isolated from different vegetables

Antibiotics	Number of *Listeria monocytogenes* susceptible or resistant to different antibiotics from different vegetables					
	Telferia occidentalis		*S. macrocarpon*		*P. soyauxii*	
	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)
Ciprofloxacin (10µg/disc)	8(58.06)	12(38.71)	8(29.62)	3(11.10)	3(12.50)	6(25.0)
Norfloxacin (10µg/disc)	10(32.25)	9(29.03)	7(25.92)	12(44.40)	4(16.60)	10(41.60)
Gentamycin (10µg/disc)	06(19.35)	18(58.06)	9(33.33)	14(51.85)	9(37.50)	12(50.00)
Lincocin (20µg/disc)	0(0)	26(83.87)	19(37.03)	6(22.22)	6(25)	18(75.0)
Streptomycin (10µg/disc)	0(0)	21(67.74)	11(40.74)	15(55.55)	1(4.20)	14(58.3)
Rifampicin (20µg/disc)	0(0)	14(45.16)	6(22.22)	10(37.03)	12(50)	9(37.50)
Chloramphenicol (30µg/disc)	0(0)	18(58.06)	4(14.81)	18(66.60)	8(53.30)	10(41.60)
Ampiclox (20µg/disc)	0(0)	10(32.25)	5(18.51)	14(14.85)	9(37.50)	12(50.0)
Floxapen (20µg/disc)	0(0)	10(32.25)	9(33.33)	12(44.40)	7(29.0)	18(75.0)
Erythromycin (30µg/disc)	0(0)	16(51.25)	19(37.03)	6(22.22)	4(14.8)	6(25)
Avicef (30µg/disc)	26(83.87)	3(9.67)	21(77.80)	5(18.51)	5(18.51)	0(0)
Siprosan (30µg/disc)	25(80.64)	3(9.67)	23(85.20)	4(14.81)	4(14.81)	1(4.20)
Drovid (30µg/disc)	31(100)	0(0)	26(96.30)	0(0)	0(0)	4(16.0)
Ciprofsin (30µg/disc)	21(67.74)	4(12.90)	22(81.50)	3(11.10)	3(11.10)	7(29.0)

Number of isolates from *Telferia occidentalis= 31*
Number of isolates from Solanum macrocarpon= 27
Number of isolates from Pterocarpus soyauxii= 24
*S; Susceptible. R; Resistan*t
Numbers in Brackets are in percentages.
Zones of inhibition < 10 are resistance markers, zones of inhibition > 21 are susceptibility markers

Similarly, *Listeria monocytogenes* was discovered 20% contaminating cabbages (Racemaekers and Lannoy, 2001).

Contamination of cabbages is also a result of the fact, that cabbages are vegetables that are in contact with soil, farmland, compost and rainwater run-off. All these materials (soil, farmland, compost) synergistically increase the bioload of the organism.

The contamination of food of animal origin must be attributed to the chains of events in the slaughter through processing, storage and preparation. Chukwu *et al.,* (2006).

Although, the minimum infective dose of *Listeria monocytogenes* is unknown, it is important to state categorically that the presence of a single cell of organism in food product is grossly intolerable. (Onwuchekwa and Okereke, 2004; Dyes and Dworaczek, 2002).

Pregnant women are advised to take vegetable diet, but another issue of health concern is the transmission of listeriosis through these recommended vegetables. Listeriosis in pregnant women has brought grave consequences to unborn foetus, and expectant mothers. (Nester *et al.,* 2004; Potter and Hotchkiss, 2006).

The major available preservative method for vegetables especially in a developing nation like Nigeria is refrigeration. This method is effective in preserving the vegetable as well as multiplication of *Listeria monocytogenes.* Nicklin *et al.,* (2003).

The result of sensitivity tests on the pathogen isolated showed that the organism has a high resistance to most antibiotic designed against gram positive organisms.

The isolates from *Telferia occidentialis* showed much resistant against streptomycin (67%), rifampicin (45%), chloramphenicol (58%), ampiclox (32%), floxapen (32%) and erythromycin (51%).

The isolates from *Solanum macrocarpon* and *Pterocarpus soyauxii* showed high level of resistance against the Aminoglycosides, β-lactam group of antibiotics. This high resistance may be as a result of acquisition of plasmid for drug resistance by *Listeria monocytogenes* strains from it natural environment such as soil, vegetables. This work is similar to the work of David *et al.,* (2006) which isolated Chloramphenicol, tetracyclin resistant *Listeria monocytogenes* from soil samples in Ado-Ekiti, Nigeria.

In Nigeria, *Listeria monocytogenes* has been isolated from ready-to-eat local beaverages such as kunu, a lactic acid fermentation product of millet or sorghum (Gaffa and Ayo, 2002; Broom, 1983; Chukwu *et al.,* 2003). The contamination of dairy products like cooked salad, suya, fura de nunu (raw milk), ice creams in Nigeria. Chukwu *et al.,* (2003), Boyle *et al.,* (1999).

The best way to manage, an infectious disease is to avoid contact with the causative agent.

In view of this, it is recommended that the use of raw fresh vegetables in preparation of food, be done with extreme hygiene caution.

The vegetables should be thoroughly washed, and pretreatment in heat (steaming, blanching) is encouraged to knock off this vegetative pathogens.

Government of Nigeria, Africa, Asia etc should intensify research and campaign with their research communities against food borne diseases such as listeriosis.

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