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Erwinia Rots and Presence of Pathogenic Bacteria in Symptomless Fruits and Vegetables

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Abstract: Spoilage and contamination of fruits and vegetables with pathogenic microorganisms constitute major agricultural and health concerns to both producers and consumers. The role played by *Erwinia* species in postharvest losses and the health associated risks in consuming fruits and vegetables without prior heat treatment were investigated. Fruits and vegetable samples collected from three municipal markets were cultured for the presence of *Erwinia* spp. and pathogenic bacteria. Pathogenicity study for *Erwinia* was carried out to demonstrate species specific predilection for certain crops. Antibiotic susceptibility of the isolated pathogenic bacteria was carried using the disc diffusion method. *Erwinia* spp. were isolated more from spoilt vegetables (80% -100%) than from spoilt fruits (65%-100%) and showed species preference for particular crop type. *Erwinia papayae* was isolated from *Carica papaya* (pawpaw) only, *E. amylovora* from *Malus domestica* (apple, 65%) and *Persea americana* (avocado pear, 100%), while *E. carotovora* was isolated from the vegetables; *Solanum tuberosum* (potato, 100%), *Solanum lycopersicum* (tomato, 90%), and *Alium cepa* (onion, 80%). Fruits and vegetables with no obvious symptoms of spoilage were contaminated with species of *Salmonella*, *Shigella*, *Escherichia*, *Klebsiella*, *Pseudomonas*, *Bacillus* and *Staphylococcus*. Multidrug resistance was found in some of the isolates especially to chloramphenicol, ciprofloxacin, erythromycin and tetracycline. Oxacillin resistance was as high as 29% in coagulase negative staphylococci. These findings underscore the importance of good hygiene and environmental sanitation in maintaining the keeping quality of fruits and vegetables postharvest.

Keywords: *Erwinia*, spoilage, fruits, vegetables, pathogens, postharvest

Introduction

Outbreaks of gastroenteritis and food poisoning have been reported in consumption of fruits or vegetables contaminated with toxigenic strains of *Bacillus cereus, Clostridium perfringens, Escherichia coli, Salmonella* spp. and *Staphylococcus aureus* [1-3]. *Erwinia* a genus in the Family Enterobacteriaceae has been implicated as major cause of postharvest rots in fruits and vegetables with serious economic consequences [4,5]. *Erwinia papayae* causes bacterial crown rot (BCR) in *Carica papaya*. Fire blight disease in apple and pear is caused by *E. amylovora* while *E. carotovora* is the cause of soft-rot diseases in carrots, potatoes, cucumbers, onions, tomatoes, lettuce, mustard and ornamental plants such as iris [6-8].

Fruits and vegetables are perishable crops with a short shelf-life [9]. Consequently, these cannot be stored in the raw form for prolong period in the fresh form without heavy chemical treatment [10,11]. The use of cold storage has limited application and is hindered by availability of constant electricity. Hygiene status of the storage facility; transportation conditions and handling, all contribute to the rapid deterioration of fruits and vegetables and entrance of pathogens. *Erwinia papayae* has been recovered from seed of infected papaya fruit and was shown to be viable in seed after an extraction and air drying process [12]. This indicates seed-borne transmission. Many peasant farmers cultivate seeds gleaned from fruits purchase from the market. This is an easy way of transmitting disease into a disease free orchard. Therefore, early detection can help prevent this occurrence. Moreover, outbreaks of gastroenteritis associated with consumption of seemingly healthy fruits or vegetables demand that frequent screening of pathogenic and spoilage organisms be carried out in order to reduce the health inherent risks by proffering methods of control [13,14]. In the present study we report on the pathogenesis of *Erwinia* species in selected fruits and vegetables and assess the health implications of consuming fruits or vegetables contaminated with pathogenic bacteria.

Materials and Methods

Culture Media

The media used in this study include: Nutrient agar, Nutrient broth, Peptone water, MacConkey agar, *Salmonella-Shigella* agar, Eosin Methylene Blue agar, Mannitol Salt agar, Mueller-Hinton agar, Simmon Citrate agar, Urea Base agar, MRVP broth, Sulphur Indole Motility (SIM) agar. These were products of BioMerieux and were prepared according to Manufacturer's instructions. Prepared media in sterile agar plates, agar slants in Bijou bottles, broth medium in sterile McCartney bottles and agar dips in screw capped test tubes were stored either at 6-8 ^{o}C or room temperature as appropriate. Sucrose Nutrient agar for the isolation of *Erwinia* spp. was prepared by adding 5 g of Sucrose to 95 ml of molten Nutrient agar and steam sterilized to give a 5% Sucrose Nutrient agar (SNA).

Antibiotics

Antibiotics used for the antibiotic susceptibility testing include; erythromycin (E, 15µg), penicillin (P, 10 IU), gentamicin (CN, 30µg), ciprofloxacin (CIP, 5µg), tetracycline (TE, 30µg), chloramphenicol (C, 30µg), oxacillin (Ox, 5µg), sulphamethoxazole/trimethoprim (SXT, 25 µg) and nalidixic acid (NAL, 30µg). The antibiotics were manufactured by

BioMerieux, France. Antibiotics discs were stored at 6-8 ^{o}C, and before use were allowed to attain room temperature (27-32 ^{o}C) before application onto the surface of inoculated Mueller-Hinton agar plates.

Table 1: Zone interpretive standards for the bacterial isolates

Antibiotics	S Interpretive Criteria (nearest whole mm)	R Interpretive Criteria (nearest whole mm)
Ciprofloxacin	≥ 21 (≥ 31-*Salmonella*)	≤15
Gentamicin	≥18 (≥15 – *Pseudomonas*)	≤12
Erythromycin	≥13	≤12
Tetracycline	≥15	≤11
Sulphamethoxazole/trimethoprim	≥16	≤10
Oxacillin	≥18	≤17
Penicillin	≥29	≤28
Chloramphenicol	≥18	≤12
Nalidixic acid	≥19	≤13

Key: S, susceptible; R, Resistant.
Clinical Laboratory Standards Institute, 2016 [15])

Collection of Samples

Two markets in Lagos (Jakande market in Ketu and Mile 12, along Ikorodu road) and Oja-Ota market Idiroko road in Ogun state) where fruits and vegetables are sold were selected for this study. Three fruits (*Malus domestica*, apple; *Persea americana*, avocado pear; *Carica papaya*, pawpaw) and three vegetables (*Solanum tuberosum*, potato; *Solanum lycopersicum*, tomato; *Allium cepa*, onion) were used for this study. Samples were collected twice weekly and over a period of 3 months. Infected and non-infected fruits and vegetables samples were collected in Ziploc bags rinsed in 2% sodium hypochlorite. The samples physical state was observed and spoilage type was recorded. Fruits with no visible sign of spoilage were classified as wholesome based on physical appearances. Healthy looking fruits but with breaks on the skin were grouped as injured fruits. Fruits or vegetable with outward healthy appearances but showed disease symptoms when pulped was recorded as inner spoilage. All spoilt samples and healthy samples within the different categories were cultured same day of sampling for the presence of *Erwinia* spp., Enterobacteriaceae, *Staphylococcus*, *Pseudomonas* and *Bacillus* spp. Three fruits (*Malus domestica*, apple; *Persea americana*, avocado pear; *Carica papaya*, pawpaw) and three vegetables (*Solanum tuberosum*, potato; *Solanum lycopersicum*, tomato; *Allium cepa*, onion) were used for this study. Samples were collected twice weekly and over a period of 3 months. A total of 30 samples of each fruit type or vegetables

were collected. Another set of healthy and disease free samples of apple, pawpaw, pear, potato, tomato, and onion were used for *Erwinia* pathogenicity study.

Processing of Samples for Culturing

The fruits and vegetables samples were rinsed with sterile water and dipped into sodium hypochlorite for surface disinfection. They were left at temperature of 16-21 ^{o}C (mean, 19 ^{o}C) in an air conditioned room to drip dry in a disinfected glass cabinet. A sterile scalpel blade was used to remove the skin (epidermal layer of the fruit and 1g of the underlining tissue was transferred into 9 ml of sterilized phosphate buffered saline in test tubes and further 10 fold serial dilutions were prepared. The fruits and vegetables were then pulped to observe the inner cavity for evidence of spoilage. Sterile scalpels were used in sampling the content of the cavity and dilutions prepared as described. Aliquots of 0.1 ml of the dilutions were inoculated onto the media for the isolation of either spoilage organisms or pathogenic bacteria. The plates were incubated at 37 ^{o}C for 18-48 h aerobically. Inoculated MacConkey agar plates, sucrose nutrient agar plates and nutrient agar plates for isolation of *Erwinia* species were incubated at two separate temperatures (28 ^{o}C and 37 ^{o}C). The streak and pour plate methods were employed. The pour plate technique was used mostly for microbial enumeration. Isolated organisms were identified by standard methods which include microscopy, cultural features and biochemical characterization.

Pathogenicity Study

The *Erwinia* isolates were assessed individually for pathogenicity. Bacterial suspension at a concentration of 1×10^{8} CFU per ml was prepared and inoculated into healthy fruits as described below. The surfaces of disease free fruits or vegetables were disinfected by swabbing with sterilized cotton swab soaked with sodium hypochlorite. The test samples were divided into two categories; wounded and unwounded samples. For wounded samples, wounding was artificially created by incision with sterile scalpel. Wounding was done to simulate the effect of breaks on fruit skin on the rate and degree of spoilage. Rectangular sterile plastic discs were soaked in the bacterial suspensions, dried and used as the source of inoculums. These were placed on the wounded and unwounded sections of the samples and stored in an air conditioned room with mean temperature of 19 ^{o}C for one set and for another at room temperature with mean of 32 ^{o}C. The progression of the disease symptoms was observed and recorded from day three.

Antibiotics Susceptibility Test

Pure culture (18-24 h) of the sample on a non-selective medium was prepared. The turbidity was adjusted to 0.5 McFarland Turbidity Standards in phosphate buffered saline (pH 7.2). The surface of Mueller-Hinton agar plates were inoculated with albumin coated swabs soaked with the inoculums. Antibiotic discs were placed on the surface of the seeded Mueller-Hinton agar and incubated aerobically at 37 ^{o}C for 18 h. Zones of inhibition were measured and interpreted against the CLSI 2016 standards [15] (Table 1).

Results

The microbial load of adhering surface flora of fruits and vegetables from the three markets after disinfection as described in this study is given in Table 2. The values indicate that samples from Jakande-Ketu market had the highest

microbial load compared with those from the other markets. Tomato had the highest microbial count 4.0 x 10^8 CFU/ml while the least count was from onion 1.8 x 10^3 CFU/ml.

Tables 3 and 4 show percentage distribution of pathogenic bacteria in the three categories of fruits and vegetables studied. Generally, fruits with inner spoilage had the highest incidence of pathogenic bacteria, followed by fruits with breaks on their skin. However, some fruits without obvious spoilage symptoms and no noticeable breaks on the skin grew pathogenic bacteria especially *Bacillus* spp. Genera in Enterobacteriaceae were predominant; however, *Shigella* was the least encountered in the fruits and vegetables.

Table 5 gives the resistance of the pathogens to some antibiotics. *E. coli* gave moderate 30.7% and high level 61.5% resistances to erythromycin and tetracycline respectively. With the exception of ciprofloxacin for which zero resistance was recorded, *P. aeruginosa* showed varied resistance to other antibiotics (8.7% for nalidixic acid and tetracycline) and 26.1% to chloramphenicol. *Klebsiella pneumoniae, Salmonella* and *Shigella* also recorded high level resistances against erythromycin and tetracycline. Ciprofloxacin resistance was seen in 83.3% *Salmonella* isolates and 66.7% *Shigella* isolates. Zero resistance to gentamicin and ciprofloxacin was recorded against the staphylococci. For other antibiotics, the coagulase negative staphylococci recorded higher resistances compared with *S. aureus*. Oxacillin resistance of 29% was seen only in coagulase negative staphylococci.

Erwinia amylovora was the only species isolated from apple (65%) and pear (100%), while *E. carotovora* was isolated from potato (100%), onion (80%) and tomato (90%). *Erwinia papayae* was isolated from 75% of pawpaw samples (Table not shown). The disease symptoms associated with the various *Erwinia* species are depicted in Figure 1. The pathogenic capacity of *Erwinia* spp. for preferred hosts is indicated in Figures 2 and 3 and plates 1-4. Disease progression and severity was more pronounced at 32 ^oC except for potato where disease severity and progression was enhanced at 19 ^oC.

Table 2: Microbial load of fruits and vegetables from different markets

Samples	*Market (CFU/ml) A	B	C
Apple	3.5 x 10^7	2.7 x 10^6	6.8 x 10^6
Pear	9.3 x 10^5	9.7 x 10^4	2.8 x10^4
Pawpaw	1.3 x 10^3	1.0 x 10^2	4.6 x 10^2
Potato	1.3 x 10^6	1.7 x 10^5	3.0 x 10^5
Tomato	4.0 x 10^8	2.6 x 10^8	2.0 x 10^8
Onions	1.8 x10^3	3.8 x 10^2	3.0 x 10^2

Keys: A, Jakande – Ketu, Lagos state; B, Mile 12, Lagos state; C, Ota, Ogun state;
*Mean count

Table 3: Distribution of pathogenic bacteria in spoilt and wholesome fruits

Isolate	Percentage distribution of bacteria in fruits								
	Apple			Pear			Pawpaw		
	IF (40)	IS (30)	WS (30)	IF (45)	IS (25)	WS (30)	IF (40)	IS (30)	WS (30)
Escherichia coli	27.5	26.7	10	26.7	36	13.3	32.5	26.7	3.3
Staphylococcus spp.	12.5	23.3	23.3	28.9	44	20	30	33.3	16.7
Staphylococcus aureus	70	56.7	16.7	48.9	60	10	30	30	6.7
Pseudomonas aeruginosa	37.5	50	10	22.2	60	6.7	32.5	23.3	3.3
*Klebsiella pneumonia*e	22.5	6.7	10	20	32	16.7	22.5	26.7	10
Enterobacter spp.	25	33.3	0	26.7	52	6.7	27.5	33.3	6.7
Salmonella spp.	17.5	26.7	16.7	6.7	36	23.3	15	30	0
Shigella spp.	7.5	0	0	4.4	0	3.3	5	0	0
Bacillus subtilis	25	5	6.7	0	15.6	44	13.3	25	13.3
Bacillus cereus	37.5	30	3.3	0	36	3.3	0	40	3.3

Key: IF, injured fruits; IS, fruits with inner spoilage; WS, wholesome fruits, *values in parentheses are numbers of samples studied

Table 4: Distribution of pathogenic bacteria in spoilt and wholesome vegetables

Isolate	Percentage distribution in vegetables								
	Potato			Tomato			Onion		
	IV (40)	IS (30)	WV (30)	IV (45)	IS (25)	WV (30)	IV (40)	IS (30)	WV (30)
E. coli	17.5	26.7	6.7	28.9	36	6.7	52	6.7	3.3
Staphylococcus spp.	20	23.3	6.7	26.7	44	26.7	0	33.3	10
S. aureus	22.5	56.7	16.7	48.9	60	23.3	12.5	30	10
P. aeruginosa	25	50	20	20	60	16.7	12.5	23.3	13.3
K. pneumoniae	22.5	6.7	0	22.2	32	10	22.5	26.7	6.7
Enterobacter spp.	7.5	33.3	6.7	26.7	52	23.3	10	33.3	3.3
Salmonella spp.	52	6.7	16.7	6.7	36	10	0	30	6.7
Shigella spp.	0	0	0	4.4	0	6.7	5	0	0
B. subtilis	25	56.7	0	15.6	44	10	25	13.3	20
B. cereus	7.5	30	0	0	36	0	0	40	13.3

Keys: IV, injured vegetables; IS, vegetables with inner spoilage; WV, wholesome vegetables; *values in parentheses are numbers of samples studied

Table 5: Resistance profile of the isolates to some antibiotics

Isolates (number)	Number (%) Resistance						
	C	CIP	CN	SXT	E	TE	NAL
E. coli (13)	0(0)	0(0)	0(0)	0(0)	8(61.5)	4(30.7)	0(0)
P. aeruginosa (23)	6(26.1)	0(0)	4(17.4)	5(21.7)	3(13.0)	2(8.7)	2(8.7)
K. pneumoniae (16)	2(12.5)	0(0)	0(0)	0(0)	16(100)	16(100)	0(0)
Enterobacter spp. (14)	0(0)	0(0)	0(0)	0(0)	7(50)	3(21.4)	0(0)
Salmonella spp. (18)	2(11.1)	15(83.3)	0(0)	0(0)	18(100)	17(94.4)	2(11.1)
Shigella (3)	0(0)	2(66.7)	1(33.3)	0(0)	3(100)	2(66.7)	1(33.3)
	CN	CIP	SXT	E	TE	OX	P
Staphylococcus spp. (31)	0(0)	0(0)	15(48.4)	3(9.6)	13(41.9)	9(29.0)	31(100)
S. aureus (25)	0(0)	0(0)	25(100)	0(0)	25(100)	0(0)	25(100)

Key: C, chloramphenicol; CIP, ciprofloxacin; N, gentamicn; E, erythromycin; TE, tetracycline; Nal, nalidixic acid; OX, oxacillin; P, penicillin; SXT, sulfamethoxazole-trimethoprim

Spoilt fruit/vegetable sample	Description	Organism isolated
	Ripe Papaya with reddish colouration and sunken lesion	*Erwinia papayae*
	Unripe papaya with sooty rots and sunken lesion	*Erwinia papayae*
	Ripe Papaya with soft rot symptoms	*Erwinia papayae*
	Affected pear fruit with water soaked appearance, wilt, shrivel and scorched	*Erwinia amylovora*
	Affected pear fruits with water soaked appearance and turn brown to black as the infection advanced	*Erwinia amylovora*
	Affected Apple fruits with sunken lesion	*Erwinia amylovora*
	Potato with soft rot and white fluffy rot symptoms	*Erwinia carotovora*
	Infected potato with inner soft rot	*Erwinia carotovora*

	Tomato with soft rot *Erwinia carotovora* symptoms
	Tomato with soft rot and water soaked lesion *Erwinia carotovora*
	Onion with inner soft rot *Erwinia carotovora* symptoms
	Onion with soft rot *Erwinia carotovora* symptoms

Figure 1: Fruits and vegetables with spoilage description and organisms isolated

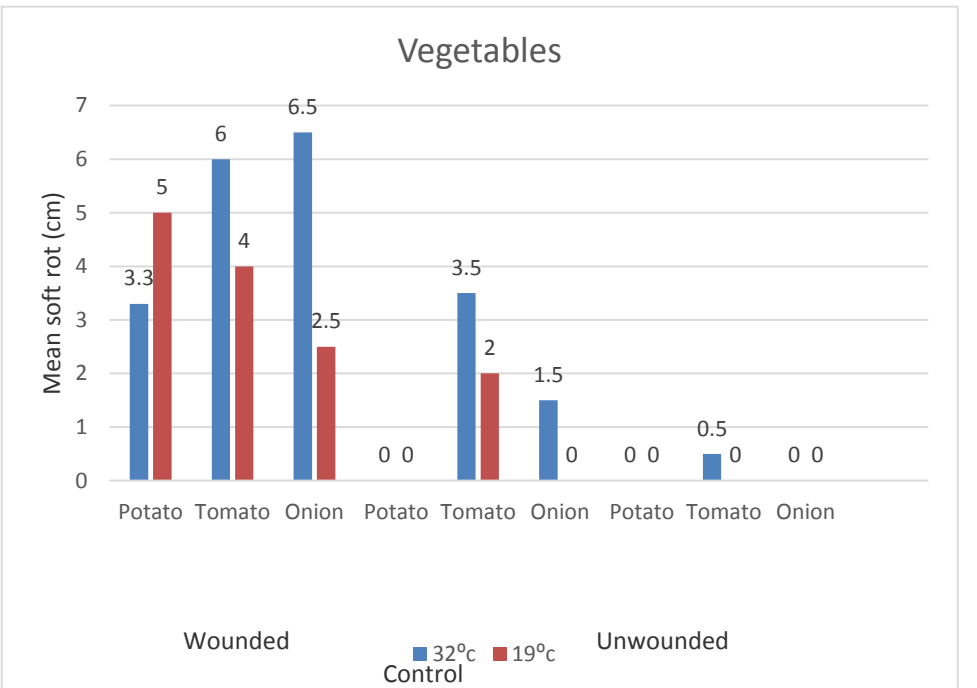

Figure 2: Diameter of diseased section of vegetables following inoculation with isolated *Erwinia* species

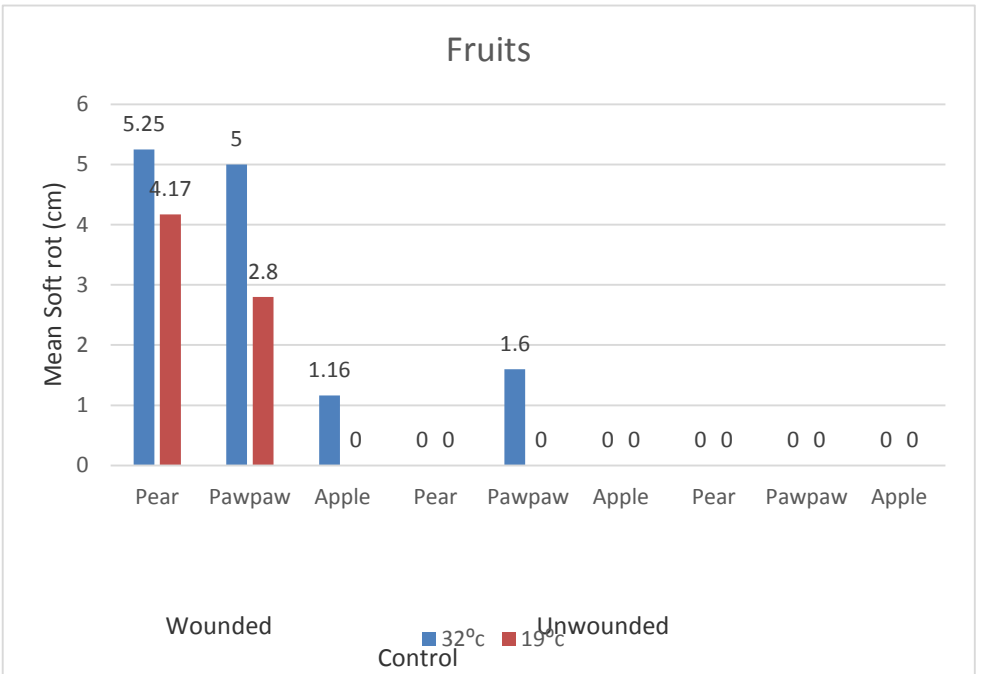

Figure 3: Diameter of diseased section of fruits following inoculation with isolated *Erwinia* species

Plate 1: Pathogenic effect of *E. amylovora* on healthy pear without artificial wounding at 32 ^{o}C three days after inoculation

Plate 2: Un-inoculated healthy pear fruit stored at 32 ^{o}C for three days

Plate 3: Disease symptoms (scorch fire appearance) caused by *E. carotovora* day five on wounded healthy potato stored at 19 ^{o}C

Plate 4: Enhanced pathogenicity of *E carotovora* on wounded healthy potato on day five after inoculation and stored 32 ^{o}C.

Discussion

Fruits and vegetables are consumed for their rich contents of energy, vitamins and minerals. These benefits can be diminished as a result of contamination with spoilage or pathogenic microorganisms. In this study, wholesome fruits and vegetables were analyzed for presence of microorganisms. The microorganisms isolated are direct reflections of the deplorable state of sanitation and hygiene of the environment and the vendors of these farm produces as evident in the high microbial counts of samples from the markets. Other contributing factors which could not be measured in this study but well documented in literature as predisposing to food contamination with microbes are processing water, harvesting techniques, transportation and storage conditions [16,17]. Since these food items are eaten in the fresh form or receive minimal heat treatment, the presence of pathogens constitutes health hazard. Postharvest rots remain a limiting factor to both the nutritional benefit and industrial use of fruits and vegetables. In the present study, *Erwinia* was observed to play a major role in the postharvest rots of fruits and vegetables; thus highlighting the agricultural significance of this bacterium.

This study demonstrated that *Erwinia* species are major postharvest spoilage organisms in fruits and vegetables. Therefore, methods of controlling their survival and proliferation in fruits and vegetables postharvest should be implemented. Available methods of control which have yielded some measurable degree of success include phytosanitation [18,19], chemical control with copper hydroxide [20], disinfection either with potassium-manganous oxide (5%) and a quaternary ammonium compound at concentration

of 10%, or sodium hypochlorite [21]. However, the health implication of the use of these chemicals in control of *Erwinia* rots in fruits and vegetables should be considered. *Erwinia carotovora* showed great predilection for potato, tomato and onion, indicating its wide host range as opposed to *E. papayae* and *E. amylovora* which have narrow host range [22]. This was further corroborated in the pathogenicity study that showed *E. carotovora* aggressiveness for both potato and pawpaw.

All the bacteria isolated in this study have previously been isolated from fruits and vegetables in other studies, both in Nigeria and elsewhere [2, 23-27]. However, the present study in addition, showed that wholesome looking fruits contained microorganisms that are of public health concern such as *Salmonella* and *Shigella*. This therefore calls for caution in the consumption of raw fruits and vegetables. *Pseudomonas* spp. and *Bacillus* spp. are part of the natural flora and are among the most common vegetable spoilage bacteria [28], though some *Bacillus* species (*B. cereus*) are capable of causing foodborne illness. The medical importance of most of the organisms isolated in this study requires that good hygiene practice and environmental sanitation be observed to safeguard the health of the consumers of farm produce.

The results for the antibiotic susceptibility test indicated that fruits and vegetables may be media for the spread of antibiotic resistant bacteria. A major finding is the resistance of a large percentage of *Salmonella* and *Shigella* isolates to ciprofloxacin a first line drug for the treatment of infections associated with these pathogens. The variation in the susceptibility of these organisms towards antibiotics may be connected to their previous exposure to the antibiotics [29]. These resistant strain especially the multi-drug resistance, are mostly implicated in epidemics [30].

Conclusion

This study has established *Erwinia* as a major postharvest spoilage organism of fruits and vegetables resulting in reduction of their market value. Secondly, the resistant pattern of the pathogens to antibiotics confirmed that drug resistant organisms can be spread through food sources. Therefore, fruit and vegetable processors should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of contamination. Also, is the need to develop new, safe and acceptable postharvest disinfestation methods; but most importantly, improving on hygiene and sanitation should be emphasized at all time. These will help farmers, marketers and consumers take the necessary precautions in preventing contamination of fruits and vegetables, thus reducing the risk of spread of infections and possible epidemics.

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References

1. Nishant, R., Kaur, P., Naveen, K. (2015). Bacteriological analysis of fresh vegetables from main market of Dehradun. *International Journal of Pharm Tech Research* 8(3):412-425.
2. Uzeh, R. E., Alade, F. A., Bankole, M. (2010). The microbial quality of prepacked mixed vegetable salad in some retail outlets in Lagos, Nigeria. *African Journal of Food Science* 3 (9): 270-272.
3. Katz, D. J., Cruz, M. A., Trepka, M. J., Sarex, J. A, Filrelle, P. D., Hammond, R. M. (2002). An outbreak of tyhpoid in Florida associated with an imported fruits. *Journal of Infectious Diseases* 186: 234-239.
4. Bhat, K. A., Bhat, S. D., Masoodi, S. A., Mir, M. Y., Zargar, S., Sheikh P. A. (2010). Studies on status and host range of soft rot disease of cabbage (*Brassica oleracea* var *capitata*) in Kashmir valley. *Journal of Phytology* 2: 55- 59.
5. Wood, M. (1998). Ubi7-new tool for potato breeders. *Agricultural Research* 2: 12-13.
6. Czajkowski, R., Grzegorz, G., van Der Wolf J. M. (2009). Distribution of *Dickeya* spp.and *Pectobacterium carotovorum* subsp. *carotovorum* in naturally infected seed potatoes. *European Journal of Plant Pathology* 125: 263-275.
7. van der Zwet, T., Beer, V. (2008). Detection of endophytic, epiphytic *Erwinia amylovora* in various pear and apple tissues. *Acta Horticulturae* 151: 69-77.
8. Gardan, L., Christen, R., Achouak, W., Prior, P. (2004). *Erwinia papaya* sp. nov., a pathogen of papaya (*Carica papayae*). *International Journal of Systematic and Evolutionary Microbiology* 54: 107-113.
9. Bhale, U. N. (2011). Survey of market storage diseases of some important fruits of Osmannabad district (M.S.) India. *Science Research Reporter* 1: 88-91.
10. Kakaew, P., Nimitheathal, H., Srilaong, V. Kanlayanarat, S. (2007). Effects of $CaCl_2$ dips and heat treatments on quality and shelf-life of shredded green papaya. *Acta Hortculturae* 764: 335-342.
11. FRAC, Fungicide Resistance Action Committee (1998). 1998 status report and recommended fungicide management guidelines. Global Crop Protection Federation, Brussels, Belgium.
12. Obrero, F. P. (2005). Bacterial crown rot of papaya in the Philippines. Proceedings of First National Symposium/Workshop on Ring Spot and other diseases of papaya in the Philippines. The Philippine Phytopathology Bureau of Plant Industry, San Andres, Malate, Manila, pp 20-23.
13. Harris, L. J., Farber, J. N., Beauchat, L. R., Parish, M. E., Suslow, T. V., Garett, E. H *et al*. (2003). Outbreaks associated with fresh produce: Incidence, growth and survival of pathogens in fresh and fresh –cut produce. Comprehensive Review.*Food Science and Safety* 2: 78-141.
14. Katz, D. J., Cruz, M. A., Trepka, M. J., Sarex, J. A., Filrelle, P. D., Hammond, R. M. (2002). An outbreak of typhoid in Florida associated with an imported fruits. *Journal of Infectious Diseases* 186: 234-239.

15.CLSI (2016). Performance 6 Standards for Antimicrobial Susceptibility
Testing, 26th; CLSI supplement M100S, Wayne, PA: Clinical and Laboratory Standards Institute.
16.Beuchat, L. R. (1996). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection* 59(2): 204-16.
17.Ray, B., Bhunia, A. K. (2007). Fundamental Food Microbiology. 4th Edition. CRC Press, USA. p 492.
18.Wills, R. B. H., McGlasson, W. E., Graham, D., Lee, T. H., Hall, E. G. (1998).Postharvest: an introduction to the physiology and handling of fruits and vegetables, 3rd edition. University of New South Wales Press, Sydney.
19.The Australian Quarantine and Inspection Service, (AQIS) (2010). Condition C18638: Seed for sowing conditions for *Carica papaya*. *AQIS ICON Database* (www.aqis.gov.au/icon32/).
20.Webb, R. R. (2008). Epidemiology and control of bacterial canker of papaya caused by an *Erwinia* spp on St. Croix, U.S. Virgin Islands. *Plant Disease* 69: 305-309.
21.Nachtigall, M., Ficke, M., Schaeffer, H. J. (2006). Fire blight control on apples by pruning measures. *Gartenbau* 33(7): 7-8.
22.Asma, A., Musharaf, A., Azra, B., Neelam, K., Sana, Z., Zahoor, A. (2015). Characterization of the causal organism of soft rot of tomatoes and other vegetables and evaluation of its most aggressive isolates. *American Journal of Plant Sciences* 6: 511-517.
23. Tambekar, D. H., Mundhada, R. H. (2006). Bacteriological quality of salad vegetables sold in Amravati City (India). *Journal of Biological Sciences* 6 (1): 28-30.
24.Omemu, A. M., Bankole, M. O. (2005). Ready-to-eat (RTE) vegetable salad: effect of washing and storage temperature on the microbial quality and shelf-life. In: Microbes as agents of sustainable development. 29th Annual conference & general meeting of the Nigerian Society for Microbiology, UNAAB, Abeokuta 6-10th November, 2005. pp. 28.
25.Adebolu, T. T., Ifesan, B. O. (2001). Bacteriological quality of vegetables used in salads.*Nigerian Journal of Microbiology* 15(1): 81-85.
26.Olayemi, A. B. (2007). Microbiological hazards associated with agricultural utilization of urban polluted river water. *International Journal of Environmental Health Research* 7(2): 149-154.
27.Dunn, R. A., Hall, W. N., Altamirano, J. V., Dietrich, S. E., Robinson-Dunn, B.,Johnson, D. R. (1995). Outbreak of *Shiegella flexneri* linked to salad prepared at a central commissary in Michigan. *Public Health Reports* 110 (5): 580-586.
28.Vanderzant, C., Splittstoesser, D. F. (1992).Compendium of methods for the microbiological examination of foods, American Public Health Association, Washington D. C.
29Buhlmann, M., Bogli-Stuber, K., Droz, S., Muhlemann, K. (2008). Rapid screening for carriage of methicillin resistant

Staphylococcus aureus by PCR and associated costs. .*Journal of Clinical Microbiology* 46(7): 2151-2154.

30. Sudershan, R. V., Naveen, K. R., Kashinath, L., Bhaskar, V., Polasa, K. (2014).Foodborne Infections and Intoxications in Hyderabad, India. Hindawi Publishing Corporation Epidemiology Research International Volume, Article ID 942961, 5 pages.

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Comparative Haematological and Serum Biochemical Evaluation of Rats Fed with Hydro-ethanolic Leaves Extracts of *Moringa oleifera* and *Telfairia occidentalis*

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***Abstract*:** The study was carried out to compare the effect of hydro-ethanolic leaves extract (MOLHE) and (TOLHE) of *Moringa oleifera* and *Telfairia Occidentalis* respectively on some haematogical and serum biochemical indices. A total of fifty wistar rats of both sexes (110-255g) were randomly allotted into five groups: A to E (n = 5). Group A (control) received water treatment equivalence. Groups B and D; C and E received 200mg/kg and 300mg/kg of TOHLE; MOLHE orally for 7 and 14 days respectively. Animals in group B and D significantly increased packed cell volume (PCV), Haemoglobin (Hb) and neutrophil levels. Group C had no significant effect on haematological indices while group E treated with 300mg/kg MOLHE showed dose dependent significant increases in PCV, Hb and lymphocyte levels in comparison with the control. Animals of both extracts recorded insignificant changes in white blood cells (WBC) compared to control. Animals in groups B and C significantly ($p<0.05$) showed increased total protein concentration at 200mg/kg while serum total cholesterol (TC) was

insignificantly ($p>0.05$) lowered in all the treatment groups compared to control. Both leaves extracts could supplement total protein requirement, lower TC level and boost haemopoeitic activities. Comparatively, TOLHE and MOLHE indicated insignificant difference in biochemical parameters analysed. However, significant ($p<0.05$) difference and increment in PCV, Hb and neutrophil levels were evident in the group treated with 200mg/kg TOLHE. Thus TOLHE may be haematologically better and /or useful in the cure of anaemia than MOLHE. In contrast, MOLHE enhanced lymphocytosis and could boost immune system more than TOLHE.

Keywords: *Moringa oleifera*, *Telfairia occidentalis*, leaves extracts, comparative effects

Introduction

Green leafy vegetables are particularly important in promoting health because of their rich sources of nutrients [1]. It has been reported that consumption of plant foods are associated with numerous health benefits rooted in their various physiological effects as a result of their phytochemical and nutritional constituents [2].Thus consumption of natural plant foods and the use of nutritional therapy and phytotherapy to improve health, prevent and treat diseases are highly recommended [3]. *Telfairia occidentalis* Hook f. and *Moringa oleifera* Lam, have unique nutritional and medicinal benefits and are widely employed in folkloric medicine to treat different health conditions including malnutrition and nutrient deficient anaemia in most underdeveloped and developing parts of the world. *Telfairia occidentalis* is a plant commonly called fluted pumpkin or guard. *Telfairia occidentalis* belongs to the tribe of Joliffiae of the subfamily Cucurbitaceae [4]. The plant is perennial, dioecious, drought tolerant and usually low thrillished and thrives well in humid climate and well drained soil. It has simple, dark green viewed leaves that are as wide as 18cm and long as 35cm [5]. Ghanaians, Cameroonians, Serria Leoneans and Nigerians are the leading producers of fluted pumpkin in West Africa. The plant is believed to have originated from the Igbos in the eastern Nigeria [6]. Fluted pumpkin leaves is good source of mineral such as Mn, Ca, Fe, Zn, K, Co, Cu, Mg; protein, vitamin A, B_2, B_4, B_{12}, C, and B_3 which are essential in human and animals. The iron content in fluted pumpkin leaves is the nutritional factor for its extensive use of the leaf extraction as blood tonic against fatique and anaemia [7]. *Telfairia occidentalis* leaves extract has hepatoprotective, anti-inflammatory; cholesterolemic properties [8]. *Telfairia occidentalis* leaf meal (TOLM) had significant effect on haematological indices in animal model [9].

However, several studies have also been documented on the nutritive, therapeutic and prophylactic potentials of *M. oleifera* leaf meal. *Moringa oleifera* Lam is the most widely cultivated species of the genus, moringa or drumstick tree is the English common name. *Moringa oleifera* (MO) is a member of the family moringaceae, a perennial angiosperm plant [10]. MO is a native of the sub-Himalaya northern part of India and is cultivated across tropical and subtropical countries of the world including Nigeria. It is a fast growing evergreen deciduous tree, which can reach a height of 10-12m and

trunk, a diameter of 45cm; with fragile branches and the leaves that build up feathery foliage [11]. MO leaves are uniquely rich in trace elements, essential amino acids, and antioxidants such as vitamin C, flavonoids, and β-carotene [12]. Leaves extract has anti-inflammatory,anti-hyerlipidimic properties; improves humoral and cellular immunity andboosts haematological indices [13,14]. However, leaves extracts of *M. oleifera* and *T. occidentalis* are often being employed in combating malnutrition and nutrient associated anaemia [15]. This current study aimed at evaluating and comparingthe effects of *T. occidentalis* and *M. oleifera* hydro-ethanolicleaves extracts on haematological and serum biochemical parameters.

Materials and Methods

Plant Material and Extracts Preparation

The fresh leaves of *Telfairia occidentalis* was bought at Oja Oba market, Owo, while the fresh leaves of *Moringa oleifera* was collected in the department of Biological Sciences, College of Natural and Applied Sciences, Achievers University, Owo, Ondo State. Both plant leaves were authenticated by a Biochemist in the department of Biological Sciences, College of Natural and Applied Sciences, Achievers University, Owo, Ondo State, Nigeria. The both leaves were washed with clean water, separately shade-dried and reduced to a powdery form by grinding using electric blender, model EM-242. In separate set up169.4 g of *M. oleifera* powdered sample and 123.7g of *T. occidentalis* were soaked in 1000ml and 770ml of 50% ethanol (hydro-ethanol) for 48 h at 4 ^{o}C respectively. After which it was sieved with a white cloth and then with Whatman No 1 filter paper (24cm). The filtrates were concentrated to complete dryness in water bath at 37 ^{o}C – 40 ^{o}C to obtain a semisolid extract of 61.42g (36.3%) *M. oleifera* leave hydro-ethanolic extract (MOLHE), and 31.3g (25.3%) *T. occidentalis* leave hydro-ethanolic extract (TOLHE). The extracts were stored at 4 ^{o}C and used for the study.

Study Animals and Experimental Design

A total of fifty adult wistar rats of both sexes, range from 110g -255g; with average weight of 158.3g were used in the study. The rats were obtained and maintained in the Animal House of the department of Biological Sciences, College of Natural and Applied Sciences, Achievers University, Owo, Ondo State, Nigeria. They were allowed to acclimatise for two weeks. Males were separated from the females to avoid possible pregnancy. The animals were housed in wire mesh cages under standard conditions (Temperature, 25-28 °C, 12 h light-dark cycles) and fed with commercial rat pelletized diet (vitafeed Ltd., Ibadan, Nigeria) and had access to water ad libitum. After the acclimatization period, the animals were allotted into two parts. Each part was divided into five groups A, B, C, D and E of five rats each. Group A served as the control and received only 1 ml distilled water treatment equivalence while group B, C, D and E were the treatment groups. Group B and D received 200mg/Kg and 300mg/Kg of the TOLHE while group C and E also received 200mg/Kg and 300mg/Kg of the MOLHE respectively. Both extracts were administered orally for 7 and 14

days. On the 8th and 15th day, rats were anaesthetised with chloroform and samples were collected for some haematological and serum biochemical parameters.

The study was generally conformed to the guidelines of the National Institute of Health for laboratory animal care and use [16] and in accordance with the principles of good laboratory procedure [17].

Determination of Haematological and Biochemical Parameters

Whole blood was collected from the heart by cardiac puncture using sterile syringe and needle. The whole blood samples were put in Ethylene di-amine tetra acetate (EDTA) treated sample tubes for haematological assay while lithium heparin sample tubes were for biochemical assay. The packed cell volume (PCV), White blood cell count (WBC), neutrophil and lymphocyte were determined by the method of Baker and Silverton [18] while Haemoglobin (Hb) was determined by the cyanomethaemoglobin method described by Cheesbrough [19]. Also, for biochemical assay, total proteins assay was done by the method of Tiez [20] and serum albumin levels assay as described by Grant [21]. Total cholesterol and triglyceride were determined by appropriate commercial kits (Randox laboratories, UK).

Data Analysis

All statistical analyses were assessed using SPSS statistical version 20.0 Software Package. Results were expressed as mean ± SEM. Differences among the groups were analyzed by one-way analysis of variance (ANOVA). Paired samples t-test was used for comparison. Values were considered statistically significant at $p<0.05$.

Results

The effects of MOLHE and TOLHE on haematological indices are shown in Tables 1 and 2 while serum biochemical parameters are in Tables 3 and 4. Results showed that group C treated with 200mg of MOLHE within 7 days recorded insignificant ($P>0.05$) increase in PCV and Hb level compared to the control in Table 1. Also insignificant ($P>0.05$) decrease in PCV and increase in Hb level were observed in 14 days compared to control (Table 2). Following the administration of 300mg MOLHE for 7 and 14 days, group E showed dose dependent significant ($p<0.05$) increases in PCV, Hb levels and lymphocyte levels compared to the control groups in tables 1 and 2.

On the other hand, group B treated with 200mg of TOLHE significantly ($P<0.05$) increased PCV but insignificant increase in Hb level within 7 days compared to the control (Table 1). But progressive significant ($p<0.05$) increases in PCV and Hb level were recorded in 14 days. Tables 1 and 2 showed significant increase in neutrophil levels in group B throughout the experimental periods compared to control. Group D significantly ($p<0.05$) increased PCV only within 7 days however, both PCV and Hb level were significantly ($p<0.05$) increased in 14 days when compared with control in Tables 1 and 2.

Table 1: Effect of MOLHE and TOLHE on some haematological parameters for 7 days

Groups	Dose	PCV (%)	Hb (g/dl)	WBC (10^9/L)	Neut. (%)	Lymph.(%)
A(Control)	--	35.3±1.5	11.8±0.5	6.13±1.1	18.8±7.7	80.5±8.0
B(TOLHE)	200mg/Kg	39.0±3.0*	13.0±1.0	3.88±1.2	28.3±9.6*	70.3±9.6
C(MOLHE)	200mg/Kg	36.5±1.6	12.2±0.5	4.01±1.0	26.3±7.7	73.8±7.7
D(TOLHE)	300mg/Kg	40.0±4.2*	13.4±1.4	3.60±8.6	15.5±3 .0	83.5±3.0
E(MOLHE)	300mg/Kg	39.3±0.9*	15.8±5.0*	5.71±1.0	19.5±2.1	90.5±2.1*

PCV = Packed cells volume, Hb = Haemoglobin concentration, WBC = white blood cells count, Neut. = Neutrophils level, Lymph. = Lymphocytes level

*indicates significant ($p < 0.05$) mean difference from control.

Table 2: Effect of MOLHE and TOLHE on some haematological parameter for14 days

Groups	Dose	PCV (%)	Hb(g/dl)	WBC(10^9/L)	Neut. (%)	Lymph.(%)
A(Control)	--	39.0±0.4	12.1± 0.2	6.76±1.9	27.5±7.3	71.3±7.4
B(TOLHE)	200mg/Kg	45.5±1.3*	15.2±0.4*	8.69±1.4	39.0±5.8*	62.5±5.6
C(MOLHE)	200mg/Kg	38.5±0.7	12.9±0.3	8.51±6.6	29.5±2.1	69.3±2.8
D(TOLHE)	300mg/Kg	43.8±1.1*	14.6±0.8*	8.01±3.1	28.8±7.9	70.8±7.9
E(MOLHE)	300mg/Kg	44.0±1.7*	14.7±0.5*	9.31±2.9	21.5±5.7	79.5±5.0*

Groups B and C recorded significant ($p<0.05$) increase in total protein levels within 7 days, while group C also recorded 40% increase in albumin level with $p<0.05$ in 14 days treatment all compared to control (Tables 3 and 4). Total cholesterol levels were insignificantly lowered ($p>0.05$) in all the treatment groups compared to the control in tables 3 and 4. Triglyceridelevel was insignificantly lowered in group B only compared to control in table 4.

Table 3: Effect of MOLHE and TOLHE on some serum biochemical parameters for 7 days

Groups	Dose	TP (g/dl)	ALB (g/dl)	TC (Mmol/L)	TRIG. (Mmol/L)
A(Control)	--	67.7±3.1	41.8±1.5	1.9±0.1	1.4±0.0
B(TOLHE)	200mg/Kg	72.1±2.2*	42.8±2.0	1.6±0.1	1.6±0.0
C(MOLHE)	200mg/Kg	72.5±1.9*	43.6±1.6	1.7±0.1	1.5±0.6
D(TOLHE)	300mg/Kg	69.9±3.6	41.9±0.8	1.7±0.0	1.4±0.0
E(MOLHE)	300mg/Kg	69.8±4.4	41.4±1.8	1.8±0.2	1.4±0.5

Table 4: Effect of MOLHE and TOLHE on serum biochemical parameters for 14 days

Groups	Dose	TP (g/dl)	ALB (g/dl)	TC (Mmol/L)	TRIG. (Mmol/L)
A(Control)	--	69.4±1.0	41.1±1.8	2.0±0.0	1.3±0.1
B(TOLHE)	200mg/Kg	72.6±2.9	42.4±1.5	1.8±0.1	1.1±0.2
C(MOLHE)	200mg/Kg	70.1±2.2	45.1±1.7*	1.0±0.2	1.4±0.1
D(TOLHE)	300mg/Kg	69.6±3.5	42.7±1.5	1.9±0.1	1.5±0.1
E(MOLHE)	300mg/Kg	72.4±1.4	41.9±1.5	1.9±0.8	1.5±0.1

TP= Total protein, ALB= Albumin, TC= Total cholesterol; TRIG= Triglyceride
*indicates significant ($p < 0.05$) mean difference from control.

Discussion

The leaf extracts of *Telfairia occidentalis* (TO) and *Moringa oleifera* (MO) are rich in protein; essential amino acids, vitamins, and minerals. The iron content in MO and TO is the nutritional factor for the extensive use of the leaves extraction as blood tonic to treat anaemia [7,22]. Iron is a necessary component of haemoglobin and myoglobin for oxygen transport. Malnutrition is the most common and wide spread cause of nutritional anaemia, such as iron deficiency anemia: a public health problem and serious problem among pregnant women and children in the developing countries. Cobalt, a constituent of Vitamin B_{12}, is essential for the maturation of erythrocyte. Vitamins B_{12} and folate are haemopoietic factors. While ascorbic acid aids in iron absorption, copper involves in iron utilization and haemoglobin formation [23].Thus Chandra *et al.* [24] observed that supplementation of haematinics (Cu, Fe, Co, folate and vitamin B_{12}) resulted in the removal of primary causes of nutritional anaemia and subsequent treatment promotes erythropoiesis in rats. MO and TO leaves are rich in natural haematinics.

Thus the significant increases in PCV and Hb levels observed in this study following oral administration of *Telfairia occidentalis*may be due to its rich nutritional contents such as essential amino acids, vitamins and trace elements [9]. This finding is consistent with the observation of Obeagu *et al*. [9] when rats were fed with 200mg/kg and 300mg/kg of fluted pumpkin leaves for 7 and 14 days. However, the insignificant ($p>0.05$) increase in WBC also observed in this study is inconsistent with the significant ($p<0.05$) increase reported by Eseyin *et al*. [8]. Neutrophil is involved in cellular immunity and fights against bacterial infection. Rich nutritional content including copper and zincn might also contribute to the significant ($p<0.05$) increase in neutrophil level recorded in this study [25].

Despite the reported high iron content in MO leaves, oral administration of 200mg/kg (MOHLE) resulted in insignificant ($p>0.05$) changes in haematological indices compared to control. This observation is also different from the significant ($p<0.05$)

haematological values recorded in group treated with similar dose 200mg/kg TOHLE. The effect may be caused by the low iron bioavailability due to the polyphenolic compounds that exist widely in MO leaves, flowers and seeds [13]. Phenol has inhibitory effect that relates to its structure; association with gallocyl and catechol groups which chelates iron and form non-bio-available polyphenol-iron complexes [27]. However the dose dependent significant increase in PCV, lymphocyte level and haemoglobin concentration following the administration of 300mg/kg MOHLE could be explained by an increase in ascorbic acid and beta-carotene, the non-heme-iron enhancers; protein intake that provides amino acids to porphyrin, globin and transferrin synthesis [28]. These significant ($p<0.05$) increments in PCV and Hb levels agree with the findings of Ujah *et al*. [15]. Again, the insignificant increase in WBC observed in this study is inconsistent with the significant increase reported by Ujah *et al*. [15]. Copper is considered to have strong effect on the immune system and is required for antibody development and lymphocytes replication [26]. However, the significant increase in lymphocytes levels observed in this study is in support of the work done by Gupta *et al* [14] that MO leaves extract contain bioactive phytochemical constituent that could enhance lymphocytosis and thus improves cellular immunity.

Results also recorded statistically insignificant ($p>0.05$) decrease in total cholesterol levels in all the treatment groups, as well as triglyceride in the group that received 200 mg/kg of MOHLE compared to control. Cholesterol is a key component of cell membranes and also necessary for the formation of hormones such as estrogen, testosterone and vitamin D. High low-density lipoprotein (LDL) cholesterol in the body is implicated in atherosclerosis. MO leaves containphytosterol such as Beta-sitosterol and can reduce intestinal uptake of dietary cholesterol [29]. Consequently, the prevention of intestinal uptake of dietary cholesterol by beta-sitosterol and quercetin might be the mechanism of the lowered cholesterol levels compared to control observed in this study. This finding agrees with the observation of Eseyin *et al*. [8]. Significant ($p<0.05$) increase in albumin level was also observed in 200mg/kg (MOHLE) when compared with the control. The increment in the total protein levels in both plants extracts compared to control may be due to the high content of crude protein presence in MO and TO leaves. This increment is in line with the previous findings reported by Ujah *et al*. [15] that MO leaves extract is a good source of supplementary protein in animals and humans.

Conclusion

From the findings of this study, *Telfairia occidental* and *Moringa oleifera* Lam. leaves extractscould boost haemopoietic activity, supplement protein requirement, and lower serum cholesterol level.Comparatively, the MOLHE and TOLHE indicated no significant difference in the biochemical parameters analysed. However, significant ($p<0.05$) difference and increment in PCV, Hb and neutrophil levels were evident in low dose 200mg/kg TOLHE. This suggests that *Telfairia occidental* leaves extract(TOLHE) may be haematologically better and /or in the

cure of anaemia than *Moringa oleifera* leaves extract (MOLHE). In contrast, MOLHE enhanced lymphocytosis while TOLHE did not; thus MOLHE could boost immune system more than TOLHE. We therefore recommend that *Telfairia occidentalis* and *Moringa oleifera* Lam. leaves be consumed simultaneously for effective nutritional and therapeutic benefits. More studies are strongly recommended to compare the effect of these two plants on haematological and serum biochemical parameters.

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References

1.Gupta, S. and Prakash, J. (2009). Studies on Indian green leafy vegetables for their antioxidant activity. Plant Foods Hum. Nutr., 64: 39-45

2.Hunter, K.J. and Fletcher, J.M. (2002). The antioxidant activity and composition of fresh, frozen, jarred and canned vegetable. Innov Food Sci. Emerg. Technol., 3: 399-406.

3.Zhao, J. (2007).Nutraceutical, nutritional therapy, phytonutrients, and phytotherapy for improvement of human health: a perspective on plant biotechnology application. Recent Pat. Biotechnol. 1(1):75-97

4.Olorufemi, A.E,Arnold, C.I.,Emmanuel, O.,Nkaima, N. and Akeem, A.(2005). Hypoglycemic activity of Telfairia occidentalis in rats. J Pharm..Biores.,2:36-42

5.Iwela E.E.J., Onychi, O. (2009). Some biochemical, haematological and histological Responses to a long-term consumption of Telfairia occidentalis supplemented diet in rat. Pak.J Nutr.,8(8):1199-1203

6.Burkett, H.M. (1968). The results of plants of West Africa. Royal Botanic Garden Kews. England, 1: 603-604.

7.Akanbi, W.B., Adebooye, C.O, Togun, A.O., Ogunrinde, J.O. and Adeyeye, S.A. (2006). Growth, herbage and seed yield and quality of *Telfairia occidentalis* as influenced by cassava peel compost and mineral fertilizer. World Journal of Agricultural Sciences, 3(4): 508-516

8. Eseyin, O. A., Igboasoiyi, A.C., Oforah, E., Ching, P., Okoli, B.C. (2005a). Effect of extract of Telfaira occidentalis leaves on some biochemical parameters in rats. Glob. J. Pure and Appl. Sci.II (1):85-87

9. Obeagu, E.I., Chikelu, I.M, Obarezi, T.N., Ogbuabor, B.N. and Anaebo, Q.B.N. (2014). Haematological Effects of Fluted Pumpkin (*Telfairia occidentalis*) Leaves in Rats. Int. J. Life Sc. Bt Pharm. Res. 3(1):2250-2313

10.Olson, M.E. (2002). Combing data from DNA sequences and morphology for a phylogeny of Moringaceae (Brassicales). Syst. Bot. 27:55-73

11.Parrota, J.A.(1993). Moringa oleifera Lam.Reseda, horseradish tree, Moringavae, Horseradish tree family.USDA Forest Service, International Institute of Tropical Forestry.(SO-ITF-SM;61).
12.Price, M.L. (2007). The marango tree. Nota Tecnica. ECHO,17391 Durrance road North fort Myers, FL 33917, USA.
13.Siddhuraju, P. and Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera Lam.*) leaves. Journal of Agricultural and Food Chemistry. 51: 2144-2155
14.Gupta, A., Guatam, M.K., Singh,R.K.,Kumar,M.V.,Rao Ch, V., Geol, R.K., Anupurba, S. (2010).Immunodulatory effect of Moringa oleifera Lam. Extract on cyclophosphamide induced toxicity in mice. Indian J.exp. Biol.48:1157-1160.
15.Ujah, O.F., Ujah, I.R., Johnson, J.T. and Oka V.O. (2013). Effect of ethanolic leaf extract of *Moringa oleifera* on haematological and biochemical parameters of rats. J. Nat. Prod. Plant Resour., 3(2):10-14
16.NIS. (1985). Care and use of laboratory animal. National Institute of Health Publication, Washington DC., Pp 85-123
17.WHO. (1998). World Health Organization Basic OECD principles of GLP. Geneva, Switzerland
18.Baker, F. J. and Silverton, R. E. and Pallister, C.J. (1985). Introduction to Medical Laboratory Technology, 6th edn. Bounty Press limited, Nigeria. Pp354-360
19.Cheesbrough, M. (2004). Haematological test. In: District LaboratoryPractice in Tropical Countries. Part 11, 2nd Edition, pp. 311-340, Cambridge University Press U.K.
20.Tietz, N.W.(1995).Clinical Guide to Laboratory Tests.3rd Edition.WB Sunders. Philadelphia PA. Pp 518-519.
21.Grant, G.H. (1987). Amino Acids and Proteins.Fundamentals of Clinical Chemistry. Tietz N.W. Editor. Third Edition, WB Saunders company Philadelphia USA,328-329
22.Ganong, W.F. (2005). A review of medical physiology. Appleton and Lange; Pp. 496
23.Davis, G.K. and Mertz, W. (1987). Copper, Trace elements in human and animal nutrition. 5th edn., Academic Press, New York. pp. 301-350.
24.Chandra, S., Chakrabarti, A., Sarker, S. and Dhara, K. (2000). Anaemia in Black Bengal goats and its chemotherapy. Indian Journal of Animal Health39 (1): 33-35.
25.Conroy, C. (2005). Participatory livestock Research- A guide, Intermediate Technology Development Group, London.
26.Burke, J.M. and Miller, J.E. (2006). Control of *Haemonchus contortus* in goats with a sustained-release multi-trace element/vitamin ruminal bolus containing copper. Vet. Parasitol., 141: 132-137
27.Brown, R.C., Klein, A., Simmons, W.K., Hurrell, R.F. (1990). The influence of Jamaican herb teas and other polyphenol-containing

beverages on iron absorption in the rat. Nutr.Res; 10:342-353.

28. Garcia-Casal, M.N., Layrisse, M., Solano, L., Baron, M., Arguello, F., Llovera, D., Ramirez, J.,Leets, I. andTropper,E.(1998). Vitamin A and β-carotene can improve nonheme iron absorption from rice, wheat and corn by humans. J. Nutr. 128:646-650.

29. Lin, X., Racette, S.B., Lefevre, M., Spearie., CA, Most, M., Ma, L., Ostiund, R.E., Jr. (2010). The effects of phytosterols present in natural food matrices on cholesterol metabolism and LDL-cholesterol: a controlled feeding trial, Eur. J. Clin. Nutr. 64: 1481 - 1487

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Prevalence and Risk Factors Associated with Human Cytomegalovirus and Human Immunodeficiency Virus Coinfection in Pregnant Women in Ilorin, Nigeria

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Abstract: Human Cytomegalovirus (HCMV) and Human Immunodeficiency (HIV) viral infection in pregnant patient is associated with high risk of maternal complications. This was a cross sectional study aimed to determine the prevalence and risk factors of HCMV and HIV mono-infections among 230 pregnant women attending ante-natal clinic of Sobi specialist hospital Ilorin, Nigeria. Data of consenting participant was collected via interviewer-administered questionnaire and clinical report form before collection of blood sample for analysis. The extracted serums were screened for HIV and CMV using rapid test-kits (Aleredetemine ^{TM}HIV1/2) and ELISA for detection of anti-CMV IgG and IgM respectively. All 230 participants were negative to HIV infection while 97.8% and 3.0% was CMV IgG and IgM positive respectively. High positivity was recorded across all age group with 15-19 having 100% anti-CMV IgG while the 25-29 and 30-34 years age group had highest anti-CMV IgM (P=0.907). 168(73.0%) of 171(74.3%) and 57(24.8%) of 59(25.7%) subjects within the parous and nulliparous group respectively were CMV-IgG positive while highest IgM positivity was noticed among the parous group (P=0.828). High CMV-IgG sero-positivity was also noticed across all level of education (P=0.700), marital status (P=0.668), types of marriage (P=0.008) and blood transfusion status (P=0.479). All the risk factors considered for HIV were very low while those for CMV had high sero-positivity for both current and past infections. The high level of CMV among pregnant women noticed in this study could be responsible for the congenital infection among newborns prevalent in the study area.

***Keywords*:** Sero-prevalence, HIV, CMV, Co-infection.

Introduction

Human Cytomegalovirus (HCMV) is a member of the Betaherpesvirinae sub-family of herpersviridae with HIV being a member of the genus Lentivirus and family Retroviridae [1]. CMV is a common viral pathogen affecting the bulk of the world's populace by early middle age [2] with 50 to 80% of adults infected at 40 years [3]. Immuno-competent individuals typically display no symptoms of infection [4] but in individuals with compromised immune system (immature and/or weakened) such as organ transplant or Acquired Immunodeficiency Syndrome (AIDS) patients, morbidity and mortality due to HCMV as a significant pathogen is hardly avoidable [5]. These individuals show different symptoms which include; spiking fever, leucopenia (decrease in white blood cell), malaise, hepatitis, pneumonia, gastrointestinal disease and retinitis [4]. HCMV is also to blame for just about 8% of infectious mononucleosis cases [6] and is also implicated in birth defects often leading to deafness and mental retardation in the foetus if the woman is infected during pregnancy [7].

The virus also spreads in households and young children in day care centers [8] with person to person transmission requiring close intimate contact with a carrier of the virus either in saliva, urine, or other bodily fluids, it can also be transmitted sexually, via breast milk, organ transplant and blood transfusion [9]. Cytomegalovirus (CMV) persists in the host for life [10] and recurrent infections are common during pregnancy constituting the majority of congenital infections in women with high prevalence of CMV-IgG [11].

In different populations, the sero-prevalence of CMV-IgG among women of reproductive age ranges from 30% to 100% [12] and the main influencing elements of CMV-IgG variability are geographical location, socioeconomic status (SES), the woman's age and parity and most importantly hygiene level [13]. The highest CMV-IgG sero-prevalence in pregnant women of 70–100% has been observed in Africa, Asia and South America [14] while pregnant women of developed countries have demonstrated lower CMV-IgG sero-prevalence levels of about 30–50% [15].

In HIV patients, HCMV reactivations and re-infections are common, and they are a major cause of congenital infection in infants worldwide [16]. In most cases of CMV infection among healthy people, infection is usually asymptomatic, with few symptoms in some cases. Therefore, for the vast majority of individuals, CMV infection is harmless. In Lagos, Nigeria it was reported that 35(14.8%) patients: 10(4.2%) males and 25(10.6%) females were positive for HCMV infection [17]. In Maiduguri, Nigeria it was reported that the sero-prevalence of anti-CMV IgG was 100% among HIV-infected patients. Having multiple sexual partners, traditional practices such as tattooing and cupping, and blood transfusion were significant risk factor among these cases [1].

CMV infection in pregnant women is of great concern because of their immune-compromised state and risk of infection to the foetus whose immune system is not fully developed. The prevalence of CMV deduced from three locations in Nigeria namely Bida (84.2%), Lagos (97.2%) and Sokoto (98.7%) among pregnant women reveals that the infection is on the increase. This study aims to provide baseline information about HCMV and HIV co-infection

among pregnant women attending ante-natal clinic in the study area which will help in management of the infection and prevent secondary spread.

Materials and Methods

Study Setting

This research was conducted at the Ante-natal Clinic of Sobi Specialist Hospital Ilorin, Kwara State, Nigeria. Ilorin, the capital of Kwara State in Nigeria is located on $8^{o}30^{o}$N $4^{o}33^{o}$E /8.500^{o}N 4.550^{o}E.

Study Population and Sample Size

The target population were pregnant women attending ante-natal Clinic of Sobi Specialist Hospital, Ilorin. The sample size derived using Fishers formula [19] was 230.

Data and Sample Collection

Interested participants were administered an informed consent form and a structured close ended questionnaire before collection of blood samples for analysis.

Assay

The blood samples were kept in an Ethylene Diamine Tetra-acetic Acid (EDTA) anti-coagulant., after collection via needle and syringe. This was centrifuged at 3000 r.p.m. for 5 minutes; the serum was harvested into clean sterile plain bottles using Pasteur pipette and pipette tips and then frozen at -20°C for assay.

HIV assay

HIV screening of the samples was achieved by using the rapid test kits (Aleredetermine^{TM} HIV1/2) following the manufacturers instruction.

CMV assay

Enzyme Linked Immunosorbent Assay method (ELISA) was used and it has been shown to be a sensitive and reliable procedure for detection of CMV antibodies with diagnostic sensitivity and specificity of 98% and ≥ 98% respectively (Rapid Labs Limited, UK). All samples were assayed for CMV-IgG and positive ones were further assayed for CMV-IgM to confirm current infections.

The manufacturer's conditions for valid assay were met and all the entire process was according to the manufacturer's specific instructions (Rapidslab UK, 2014)

Data Analysis

The data from the questionnaire was analyzed using X^2 (chi-square) variable while test for statistical association was done using SPSS version 17.0.

Ethical Considerations

Approval for the study was obtained from Ethical Committee of Ministry of Health, Ilorin. Informed consents were obtained from patients and the study was at no cost to the subjects. Information from the patient was held confidential.

Results

Out of the 230 participants, no case of HIV infection was found and analysis of its risk factors showed that 7.83% had undergone surgery while most respondent (92.17%) had not, 4.8% have had tattoo as against those (95.2%) who did not, while few respondents (10.40%) had history of blood transfusion. Based on multiple sex partners, 22.10% of the population had multiple sex partners while 76.10% had single partners.

From a total number of 230 samples that were screened for CMV-IgG, 225 (97.8%) tested positive, 5 (2.2%) were negative and none was equivocal while IgM assay for CMV-IgG positive subjects results show that 7(3.1%) were positive, 217(96.4%) were negative and 1(0.4%) was equivocal.

All the 8(3.5%) subjects that were single and 217(94.3%) of 222 (96.5%) that were married tested positive CMV-IgG (P=0.668) while 6(2.7%) of 217(96.4%) married subject and 1(0.4%) of the 8(3.6%) single subject were positive when assayed for CMV-IgM antibodies (P=0.293). The only equivocal result was recorded among the married women.

The educational level of the respondent revealed that 9(3.9%) subjects without any form of education, 40(17.4%) of 42(18.3%) that attended only primary school, 79(34.3%) of 81(35.2%) with secondary education and 92(40.0%) of 93(40.4%) subjects that had tertiary education were all positive to CMV-IgG (P=0.700). Out of the 79 (35.1%) subjects in the secondary education group, only 1(0.4%) was positive while 3(1.3%) of 40(17.8%) subjects in primary group and 3(1.3%) of 92(40.9%) subjects in tertiary group were positive to CMV-IgM antibodies (P=0.373).

The CMV IgG and IgM status of subjects in relation to the parity status revealed that 168(73.0%) out of 171(74.3%) subjects within the parous group and 57(24.8%) of 59(25.7%) subjects in nulliparous group, tested positive to CMV-IgG (P=0.458) while results from CMV-IgM assay revealed that 5(2.2%) out of 168(74.7%) subjects within the parous group and 2(0.9%) out of 57(25.3%) subjects that are nulliparous were positive. One equivocal result was recorded among subjects in the parous group (P=0.828).

Table 1: Cytomegalovirus IgG and IgM prevalence in relation to age

		Age(yrs)									
		15-19	20-24	25-29	30-34	35-39	40-44	NO INDICATION	Total (%)	P value	X^2
IgG	POSITIVE (%)	6(2.6)	36(15.7)	102(44.3)	50(21.7)	21(9.1)	6(2.6)	4(1.7)	225(97.8)	0.883	2.371
	NEGATIVE(%)	0(0.0)	1(0.4)	1(0.4)	2(0.9)	1(0.4)	0(0.0)	0(0.0)	5(2.2)		
	Total (%)	6(2.6)	37(16.1)	103(44.8)	52(22.6)	22(9.6)	6(2.6)	4(1.7)	230(100)		
IgM	POSITIVE (%)	0(0.0)	1(0.4)	3(1.3)	3(1.3)	0(0.0)	0(0.0)	0(0.0)	7(3.1)	0.907	6.172
	NEGATIVE(%)	6(2.7)	35(15.6)	99(44.0)	46(20.4)	21(9.3)	6(2.7)	4(1.8)	217(96.4)		
	EQUIVOCAL(%)	0(0.0)	0(0.0)	0(0.0)	1(0.4)	0(0.0)	0(0.0)	0(0.0)	1(0.4)		
	Total (%)	6(2.7)	36(16.0)	102(45.3)	50(22.2)	21(9.3)	6(2.7)	4(1.8)	225(100.0)		

P value < 0.05 is statistically significant
X^2—chi square value

Table 2: Sero-prevalence of CMV IgG and IgM in relation to marriage type of respondent

		MARRIAGE TYPE			Total(%)	Pvalue	X^2
		MONOGAMY	POLYGAMY	NO INDICATION			
IgG	POSITIVE(%)	166(74.8)	48(21.6)	3(1.4)	217(97.7)	0.008	9.587
	NEGATIVE(%)	3(1.4)	1(0.5)	1(0.5)	5(2.3)		
	Total(%)	169(76.1)	49(22.1)	4(1.8)	222(100.0		
IgM	POSITIVE(%)	5(2.3)	2(0.9)	0(0.0)	7(3.2)	0.967	0.565
	NEGATIVE(%)	160(73.7)	46(21.2)	3(1.4)	209(96.3)		
	EQUIVOCAL(%	1(0.5)	0(0.0)	0(0.0)	1(0.5)		
	Total(%)	166(76.5)	48(22.1)	3(1.4)	217(100.0		

P value < 0.05 is statistically significant
X^2—chi square value

Table 3: Sero-prevalence of cytomegalovirus in relation to blood transfusion of subjects

		BLOOD RANSFUSION		Total (%)	P value	X^2
		YES	NO			
IgG	POSITIVE(%)	23(10.0)	202(87.8)	225(97.8)	0.479	0.500
	NEGATIVE(%)	1(0.4)	4(1.7)	5(2.2)		
	Total(%)	24(10.4)	206(89.6)	230(100.0)		
IgM	POSITIVE(%)	1(0.4)	6(2.7)	7(3.1)	0.886	0.241
	NEGATIVE(%)	22(9.8)	195(86.7)	217(96.4)		
	EQUIVOCAL(%)	0(0.0)	1(0.4)	1(0.4)		
	Total(%)	23(10.2)	202(89.8)	225(100.0)		

value < 0.05 is statistically significant
X^2—chi square value

Discussion

From this study, no case of HIV infection was found among the subjects and this could be due to low prevalence rate of HIV patient and its risk factors in the study location. Low prevalence of 0.7% and 5.4% in other parts of Nigeria was reported [20] in Abeokuta (South western Nigeria) and [21] in Abakaliki (South eastern Nigeria). In

concurrence with this research, a study in Kabul [22] found no case of HIV infection in the 4,452 pregnant Afghan women and another in Malekan [23] also found no case of
HIV infection in the 680 pregnant Iranian women. There is a wide geographical variation in the sero-prevalence of HIV infection amongst pregnant women within and outside Nigeria. The variation may be a reflection of the differences in sexual practices and behaviour, awareness of HIV infection and status, socio-cultural practices, and accessibility to healthcare. The level of HIV awareness and status among this group is encouraging as risk of infection to the child is been checked. Among other risk factors to HIV transmission such as surgical procedure, tattoo practice etc, low blood transfusion level was also recorded and this is in contrary to a report [24] were pregnant women with history of blood transfusion had higher prevalence of HIV infection. Thus, it can be said that the transfusion in this case was thoroughly screened for HIV. Also, the population of respondents that share multiple sex partners was low which correlates to a report [25] that exposure to multiple sex partners has been found to be associated with increased risk of acquiring HIV infection. Another study [24] also reported that high prevalence of HIV among pregnant women can be linked to involvement with multiple sex partners.

The results obtained from this study show that the sero-prevalence of Cytomegalovirus (CMV) infection among pregnant women in Ilorin, Kwara state is high with IgG 97.8% and IgM 3.1% detected. The high prevalence rates could be due to lower socioeconomic status and poor hygienic conditions of the respondents in the study location. This result is in agreement with a study [26] that had prevalence rate of 97.2% among pregnant women in Lagos, Nigeria. Also in two similar studies, [27], [28] prevalence of 97.2% in Benin and 96% in Egypt were recorded respectively among pregnant women. However, in some European countries, low CMV infection rates have been reported, Australia (56.9%) and France (46.8%) [29]. The low prevalence rates in these countries could be due to the inclusion of CMV screening among the antenatal profile tests and better hygienic standards. The detected 97.8% of CMV infections in the current study showed that these women were at higher risk of HIV infections since the risk factors are similar.

According to this study, the prevalence of CMV-IgG varied with age. The distribution of the age of the surveyed respondent showed that majority of respondent were aged 20-34years, accounting for 81.7% of the total participants and fewer percentages accounted for the older group. This is largely due to the fact that majority of pregnant women attending Ante-natal clinic belong to the child bearing age and are largely embedded in this age group. This statement conforms to study on changing pattern of cytomegalovirus sero-prevalence among pregnant women in Norway who reported that the highest CMV-IgG sero-positivity rate was detected in young women [30]. This is in contrast to other studies, reporting an increased CMV-IgG sero-prevalence with increase in age [31].

Evidence of current cytomegalovirus infection via IgM also supports that most infection occurs early in life because from the seven subjects with current infection (i.e IgM positive) as at the time of this research work four were below age 30 while three were below 35. However, there was no statistical significant (P=0.907) between CMV infection and age of the respondent. From this study, it was observed that a larger percentage of study respondents were parous (73.0%) as compared to those that were nulliparous. However, there was no significant difference (P=0.828) to the risk of
acquiring CMV infection between parous and nulliparous in the study. The result is similar to that reported [6] in Lagos state, which reported that there was no significant relatedness to the risk of acquiring CMV infection in the parity status of respondents to his study. Conversely, this in contrast to another research [29] in Kenya, which reported that there was a statistical correlation (P=0.0001) to the risk of acquiring CMV infection in the parity status correspondent to his study. According to the present data, it can be concluded that the risk of infectivity increases as the educational level gets higher because contact rate also increases which can be due to the low cleanliness level common across the group. It was also noticed that majority of the respondents in tertiary level were positive to cytomegalovirus IgG which may be due to the contact at earlier stages of education. This statement conforms to the report that illiterate women are at higher risk of CMV infection due to contact with contagious secretions from their own children and poor hygienic practice [32]. However, analysis of the result by educational level shows that there was no significant association (P=0.373) between CMV infection and educational status.

The percentage of marital survey shows that most of the respondents are married with only 3.5% of the population been single. Cytomegalovirus infection is higher in married women than in singles which suggest continuous circulation of the virus within the area and this conforms to a study that also recorded increased sero-positivity among married women than single ladies [26]. However, there was also no significant difference (P=0.294) in the transmission of CMV infection between those that were married or single in this study [17] which is in concordance with another report that there was variation in prevalence of HCMV in different marital status, but the variation was not statistically significant (P>0.05). The study showed (P=0.008) a significant association between Cytomegalovirus infection and type of marriage. With respect to the fact that majority of respondents claimed to be married, a vast majority of respondents were monogamous when compared to their counterparts whose marriage type was polygamous. The sero-prevalence of Cytomegalovirus was higher in women who had been involved in up to two marriages in their life time. This is in agreement with a research that states that the distribution of CMV sero-prevalence among pregnant women by number of marriages shows that women who had been involved in up to three marriages in their life time had the highest sero-prevalence rate [33].

There was no association between women who had been transfused and those that were not (P=0.479). The reason could be as a result of the

disproportionate size of women who were transfused to those who were not transfused enrolled in the study. This is in concordance with a research [26] that stated that past history of blood transfusion was found to be insignificant to the risk of acquiring CMV infection in his study but this statement is in contrast to a report that pointed blood transfusion as a risk factor for transmission of CMV infection [34].

Although most of the considered risk factors were not of statistical significance and there was no recorded case of co-infections, this study however shows that CMV is highly associated with pregnant women in Ilorin implying that there might be a corresponding increase in the incidence of congenital CMV infection among infant born in the state in form of CMV-related hearing loss, vision loss and poor mental development among children in Ilorin, Kwara state. Thus, increased public awareness

about CMV and HIV infection is needed across Nigeria, and this can be done through primary health care channels such as antenatal clinics where the transmission, consequences of infection on foetus and its control and preventive measures can be discussed. Efficient blood screening for CMV and HIV before transfusion to pregnant women, routine screening of pregnant women for CMV should be adopted in all health care settings and babies born to sero-positive mothers should be screened and examined immediately after delivery for possible signs of hearing and vision defect for early management.

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References

1.International Committee on Taxonomy of Viruses. (2002) "61. Retroviridae". National Institutes of Health. http://eol.org/pages/67526/details Accessed April 5th 2016.

2.Mocarski, E.S., Shenk, T. and Pass, R.F. (2007) Cytomegaloviruses. Edited by: Knipe, D. M., Howley, P. M,. Fields virology 5th edition. Philadelphia, P. A: Lippincott Williams. 2701-2772.

3.Selinsky, C., Luke, C., Wloch, M., Geall, A. and Hermanson, G. (2005). A DNA-based vaccine for the prevention of human cytomegalovirus-associated diseases. *Hum Vacc*. 1:16-23.

4.Soderberg-Naucler, C. (2006). Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer? *J Intern Med*. 2006; 259: 219-246.

5.Steininger C. (2007). Clinical relevance of cytomegalovirus infection in patients with disorders of the immune system. *Clin Microbiol Infec*. 13: 953-963.

6.Grosse, S.D., Ross, D.S. and Dollard, S.C. (2008). Congenital cytomegalovirus (CMV) infection as a cause of permanent bilateral hearing loss: a quantitative assessment. *J Clin Virol*. 41: 57-62.

7.Koch, S., Solana, R., Dela, R.O. and Pawelec, G. (2006) Human cytomegalovirus infection and T

cell immunosenescence: a mini review. *Mech Ageing Dev*. 127: 538-543.

8.Ryan, K.J. and Ray, C.G. (2004). (editors). Sherris Medical Microbiology (4th ed.). McGraw Hill.

9.Taylor, G.H. (2003). "Cytomegalovirus" *Am Fam Physician*. 67 (3): 519–24. PMID 12588074.

10.Stagno, S. (2001). Cytomegalovirus. In: Remnington, J. S., Klein, J. O. editors, eds. Infectious diseases of the fetus and newborn infant. 5th edition Philadelphia, P. A: W.B Saunders Co. 389– 424

11.Mussi-Pinhata, M.M., Yamamoto, A.Y. and Moura Brito, R.M. (2009). Birth prevalence and natural history of congenital cytomegalovirus infection in a highly seroimmune population. *Clin Infect Dis*. 49: 522–8

12.Knowles, S.J., Grundy, K. and Cahill, I. (2005). Low cytomegalovirus sero-prevalence in Irish pregnant women. *Irish Med J*. 98: 210–12

13.Gratacap-Cavallier, B., Bosson, J.L. and Morand, P.K. (1998). Cytomegalovirus seroprevalence in French pregnant women: parity and place of birth as major predictive factors. *Eur J Epidemiol*. 14: 147–52

14.Hoshiba, T., Asamoto, A. and Yabuki, Y. (1998). Decreasing seropositivity of cytomegalovirus of pregnant women in Japan. *Nippon Rinsho*. 56: 193–6

15.Marshall, G.S. and Stout, G.G. (2005). Cytomegalovirus seroprevalence among women of childbearing age during a 10-year period. *Am J Perinatol*. 22: 371–6

16.Mussi-Pinhata, M.M., Yamamoto, A.Y., Figueiredo, .LT., Cervi, M.C. and Duarte, G. (1998). Congenital and perinatal cytomegalovirus infection in infants born to mothers infected with human immunodeficiency virus. *J Pediatr*. 132: 285-90

17.Akinwale, O.P., Afilaka, B., Gyang, VP, Adeleke, M.A., Adeneye, A., Onwujekwe, D., Alimi, F. and Akande, D.O. (2013). Human cytomegalovirus infection in Nigerians living with human immunodeficiency virus. *Annu Trop Med Public Health*. 6: 59-64.

18.Ibrahim, M.K., Ballah, A.D. and Bukar, B. (20140. Seroprevalence of and Risk Factors for Cytomegalovirus among HIV-Infected Patients. *Open J Immunol*. 4: 54-6.

19.Araoye, M.O. (2004). Research methodology with statistics for health and social science. Sample size determination. Nathadex publishers: 118-21.

20.Fawole, A.O., Sotiloye, O.S., Hunyinbo, K.I., Fawole, O.I., Oladimeji, A.O. and Durodola, A. (2002). HIV in pregnancy: Experience at Abeokuta, Nigeria. *Trop J Obstet Gynaecol*. 19(1): 21-24.

21.Obi, S.N. (2005). Pregnancy outcome in HIV seropositive women in Abakaliki, Nigeria. *Orient J Med*. 17(3&4): 25-30

22.Todd, C.S., Ahmadzai, M., Atiqzai, F., Miller, S., Smith, J.M. and Ghazan, S.A. (2008). Seroprevalence and correlates of HIV, Syphilis, and hepatitis B and C virus among intrapartum patients in Kabul, Afghanistan. *BMC Infect Dis*. 8: 119.

23.Sahaf, F., Tanomand, A., Montazam, H. and Sany, A.A. (2007). Seroprevalence of Hepatitis C, Hepatitis B and HIV and co-infection among pregnant women: aretrospective study in 2006 at Malekan city, Iran. *Res J Med Sci*. 1(2): 138-141

24.Oladeinde, B.H., Omoregie, R., Olley, M. and Anunibe, J.A. (2011). Prevalence of HIV and anaemia among pregnant women. *N Am J Med Sci*. 3: 548-551.

25.Douglas, K., Robyn, D., Kelly, L. and Allison, P. (2012). Promoting partner reduction helping young people understand and avoid hiv risks from multiple partnerships. FHI 360. ISBN: 1-933702-99-0

26.Akinbami, A.A., Akinsegun, A., Kabiru, A.R., Adeniyi, A.A. and Kikelomo, O.W. (2011). Seroprevalence of cytomegalovirus antibodies amongst normal pregnant women in Nigeria. *Int J Wom Health*. 423-428.

27.Rodier, M.H., Berthonneau, J., Bourgoin, A., Giraudeau, G., Agius, G., Burucoa, C., Hekpazo, A. and Jacquemin, J.L. (1995). Seroprevalences of Toxoplasma, malaria, rubella, cytomegalovirus, HIV and treponemal infections among pregnant women in Cotonou, Republic of Benin. *Acta Trop*. 59(4):271–7. doi: 10.1016/0001-706X(95)00087-U

28.El-Nawawy, A., Soliman, A.T., el-Azzouni, O., Amer el-S, Karim, M.A., Demian, S., el Sayed, M. (1996). Maternal and neonatal prevalence of toxoplasma and cytomegalovirus (CMV) antibodies and hepatitis-B antigens in an Egyptian rural area. *J Trop Pediatr*. 42(3): 154–7. doi: 10.1093/tropej/42.3.154.

29.Zakayo, M. and Anthony, K.N. (2014). Seroprevalence of Cytomegalo Virus (CMV) among pregnant women in Thika, Kenya BMC Res Notes. 7(1): 794.

30.Odland, M.L., Strand, K.M., Nordb, S.A., Forsm, O.S., Austgulen, R. and Ann-chalotte, S. (2013). Changing patterns of cytomegalovirus seroprevalence among pregnant women in Norway. *BMJ*. Doi: 10.1136/bmj open- 003066.

31.Gratacap-Cavallier, B., Bosson, J.L. and Morand, P.K. (1998). Cytomegalovirus seroprevalence in French pregnant women: parity and place of birth as major predictive factors. *Eur J Epidemiol*. 1998;14:147–52

32.Hamdan, H.Z., Abdelbagi, I.E., Nasser, N.N. and Adam, I. (2011). Seroprevalence of Cytomegalovirus and Rubella among pregnant women in Western Sudan. *Virol J*. 18:217-218

33.Yeroh, M., Aminu, M. and Musa, B. (2015). Seroprevalence of cytomegalovirus infection amongst pregnant women in Kaduna state. *Afr J Clin Exp Microbiol*. 16(1): 37-44.

34.Matos, S.B., Meyer, R. and Lima, W. (2010). Seroprevalence of Cytomegalovirus infection among healthy blood donors in Bahia State, Brazil. *Revista Brasileira de Hematologiae Hemoterapia*. 3(1): 1516-8484

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Confirmation of Climate Change in Southwestern Nigeria through Analysis of Rainfall and Temperature Variations over the Region

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Abstract: Understanding the variability of precipitation and temperature of a region over a long period gives one an idea about the climate and climate change of such region. The study investigated rainfall and temperature variability in four meteorological stations, namely, Abeokuta (lat.7.01^o N, long.3.2^o E, alt. 67m) , Ibadan (lat.7.43^o N, long.3.9^o E, alt. 227m), Ikeja (lat.6.58^o N, long.3.33^o E, alt. 39m) and Ondo (lat.7.1^o N, long.4.83^o E , alt. 287m) in the south-western region of Nigeria.Monthly rainfall, minimum temperature and maximum temperature data were obtained from Nigeria Meteorological Agency (NIMET) for the period of thirty one years (1980 to 2010) for the study.Descriptive Statistics were deployed to determine the mean, confidence levels, coefficient of kurtosis, skewness and coefficient of variations. A fairly "M-shaped" pattern was observed in the monthly mean rainfall distribution with bi-modal peaks in June and September, with slight dryness experienced in August, referred to as "August break". Analyses of annual trends over a long period revealed a sequence of alternately decreasing and increasing trends in mean annual rainfall and air temperature in region. Generally however, gradients of the trend lines are positive. There is a negative relationship between annual total rainfall and annual average temperature.

Keywords: Climate change, statistical analysis, rainfall, temperature

Introduction

Climate is the average weather over a long period of many years. It differs in regions of the world. Climate depends on different amounts of sunlight received in a region and different

geographic factors, such as proximity to oceans and altitude. Climate is typically described by the statistics of a set of atmospheric and surface variables, such as temperature, precipitation, wind, humidity, cloudiness, soil moisture, sea surface temperature, and the concentration and thickness of sea ice. The statistics may be in terms of the long-term average, as well as other measures such as daily minimum temperature, length of the growing season, or frequency of floods. One can have a good understanding of the climate of a location by examining the annual or seasonal averages of two climatic variables: temperature and precipitation.

Climates will change if the factors that influence them vary. In order to change climate on a global scale, either the amount of heat that is let into the system changes, or the amount of heat that is let out of the system changes. The sun provides the energy that drives the climate of the earth. Variations in the composition and intensity of incident solar radiation hitting the earth may produce changes in global and regional climate which are both different and additional to those from man-made climate change.

The Intergovernmental Panel on Climate Change (IPCC) defines climate change as: A change in the state of the climate that can be identified by changes in the mean and/or the variability of its properties, and that persists for an extended period, typically decades or longer. It refers to any change in climate over time, whether due to natural variability orbecause of human activity [1]. The global climate has changed rapidly with the global mean temperature increasing by 0.7 ^{o}C within the last century [1].

Several studies have been carried out in different part of the globe in relation to climatic variations [2-5]. Ayansina and Ogunbo [3] investigated the seasonal rainfall variability in Guinea savannah part of Nigeria and concluded that rainfall variability continues to be on the increase as an element of climate change. Hasanean [4] examined trends and periodicity of air temperature from eight meteorological stations in the east Mediterranean and observed positive significant trends in Malta and Tripoli, and negative trend in Amman. Turkes *et al*. [5] evaluated mean, maximum and minimum air temperature data in Turkey during the period 1929–1999. Their analyses revealed spatiotemporal patterns of long-term trends, change points, and significant warming and cooling periods. Increasing flood risk is now being recognized as the most important sectorial threat from climate change in most parts of the region which has prompted public debate on the apparent increased frequency of extreme, and in particular, on perceived increase in rainfall intensities [6]. Several studies have adduced extreme rainfall to be the major cause of flood worldwide [7-10].

This work is aimed at investigating the pattern of rainfall and temperature, as well as the relationship between them, in four meteorological stations in south-western region of Nigeria on monthly and annual basis.

Methodology

Monthly rainfall, minimum temperature and maximum temperature data for thirty one years (1980 to 2010) of four meteorological stations were collected from Nigeria Meteorological Agency

(NIMET). The stations are, Abeokuta(lat.7.01^{o} N, long.3.2^{o} E, alt. 67m) Ibadan (lat.7.43 ^{o} N, long.3.9 ^{o} E, alt. 227m) , Ikeja (lat.6.58 ^{o} N, long.3.33 ^{o} E, alt. 39m) and Ondo (lat.7.1 ^{o} N, long.4.83 ^{o} E , alt. 287m). The stations fall into two climatic regions namely costal (Abeokuta, Ikeja and Ondo) and derived savannah (Ibadan). From the collected data, annual rainfall and annual mean temperature for the stations were calculated. The monthly and annual distributions of rainfall and temperature characteristics across each of the stations were observed and analyzed having taken care of some few incomplete and missing records. Descriptive statistics were used for the analyses. Thus, the mean, minimum, maximum, Kurtosis and skewness of the climatic variables considered were analyzed both on monthly and annual basis. The mean gave information about the centre of the distribution. The coefficients of skewness and kurtosis provided information about the symmetry and length of the tail for certain types of distributions respectively. Skewness was a measure of asymmetry of the probability distribution of a variable from mean. It revealed to us the amount and direction of departure from horizontal symmetry. It can be positive and negative or even undefined. If skewness is zero, the data are perfectly symmetrical.

The following are the general rules of skewness:

- If skewness is less than -1 or greater than 1, the distribution is highly skewed.
- If skewness is between -1 and - 0.5 or between 0.5 and 1, the distribution is moderately skewed.
- If skewness is between -0.5 and 0.5, the distribution is approximately symmetric.

The following are the general rules of kurtosis:

- If kurtosis is greater than 3, the distribution is highly leptokurtic i.e. it is sharper than normal distribution, with values concentrated around the mean and thicker tails. This signifies high probability for extreme values.
- If kurtosis is less than 3, the distribution is platykurtic i.e. it is fatter than normal distribution with a wider peak. The probability of extreme values is than for a normal distribution, and the values is wider spread around the mean.
- If kurtosis is 3, the distribution is mesokurtic i.e. it is a normal distribution.

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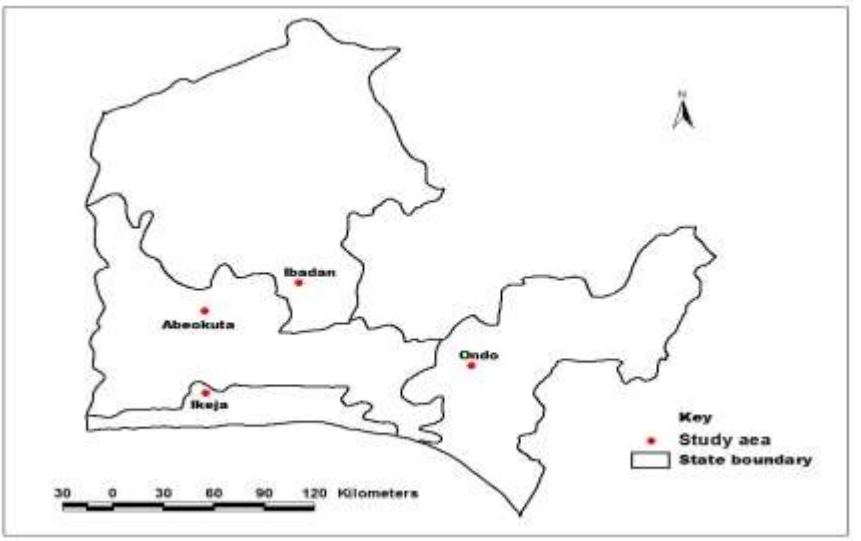

Figure 1: Location of the four meteorological stations on the southwestern map of Nigeria

Results and Discussion

Table 1: Descriptive statistics of the rainfall data for Abeokuta in coastal region

Abeokuta	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Mean	3.58	25.75	65.91	120.49	157.47	196.09	183.82	113.32	197.59	125.51	18.79	8.95
Std. Dev.	7.18	31.21	48.11	54.09	63.21	78.22	93.32	85.23	74.94	51.48	21.78	19.64
Samp. Var	51.60	974.01	2314.20	2926.26	3995.83	6118.17	8708.21	7263.84	5616.62	2650.41	474.36	385.90
Kurtosis	8.48	-0.17	1.03	1.48	1.23	2.06	0.28	-0.08	0.14	0.18	2.42	5.76
Skewness	2.71	1.01	1.08	1.20	1.01	1.12	0.90	0.80	0.79	-0.31	1.59	2.52
Range	32.50	99.20	198.90	212.20	272.30	385.30	369.20	297.30	287.90	208.20	88.00	71.70
Minimum	0.00	0.00	1.60	45.50	69.40	51.00	46.10	8.00	84.80	8.90	0.00	0.00
Maximum	32.50	99.20	200.50	257.70	341.70	436.30	415.30	305.30	372.70	217.10	88.00	71.70
Count	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	29.00	30.00	30.00
C/Lev.(95.0%)	2.68	11.65	17.96	20.20	23.60	29.21	34.85	31.82	27.98	19.58	8.13	7.34

Table 2: Descriptive statistics of the rainfall data for Ibadan in derived savannah region

Ibadan	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Mean	4.33	37.47	81.13	123.82	160.44	191.13	179.44	143.98	193.30	182.19	24.12	8.96
Std. Dev	8.42	45.45	52.83	45.56	54.07	81.51	87.58	96.21	64.26	53.78	30.24	17.46
Samp. Var.	70.90	2065.91	2790.63	2075.54	2923.21	6643.98	7669.42	9256.35	4129.94	2892.12	914.54	304.73
Kurtosis	3.02	1.45	0.60	1.02	-0.59	-0.62	-0.25	0.45	-0.50	2.56	6.50	5.71
Skewness	2.02	1.48	0.26	-0.02	0.13	0.32	0.21	0.86	0.57	0.46	2.22	2.41
Range	30.10	165.50	200.40	164.20	213.90	311.80	359.60	381.50	235.80	278.00	139.80	71.40
Minimum	0.00	0.00	0.00	39.50	51.30	61.60	26.20	29.80	92.70	70.00	0.00	0.00
Maximum	30.10	165.50	200.40	203.70	265.20	373.40	385.80	411.30	328.50	348.00	139.80	71.40
Count	30.00	29.00	30.00	30.00	29.00	29.00	30.00	30.00	29.00	30.00	30.00	29.00
C/Lev(95.0%)	3.14	17.29	19.73	17.01	20.57	31.00	32.70	35.93	24.44	20.08	11.29	6.64

Table 3: Descriptive statistics of the rainfall data for Ikeja in coastal region

Ikeja	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Mean	14.03	29.53	71.94	137.02	193.19	296.58	190.82	92.71	185.98	152.76	77.46	25.51
Std Dev.	32.72	39.02	40.53	80.18	66.28	112.12	146.37	92.75	91.78	63.46	48.64	28.45
Samp.Var.	1070.50	1522.20	1642.59	6428.39	4393.41	12571.21	21423.21	8602.49	8422.99	4027.72	2366.17	809.37
Kurtosis	21.22	9.34	-0.68	0.49	-0.19	0.82	0.70	4.63	-0.11	1.80	0.81	-0.39
Skewness	4.35	2.66	0.16	1.03	0.42	0.87	1.28	1.88	0.56	1.07	1.00	0.94
Range	174.40	188.50	150.00	309.90	265.20	484.60	518.30	415.00	370.70	305.40	207.80	87.70
Minimum	0.00	0.00	5.80	26.40	88.60	134.10	48.70	4.10	29.20	37.30	1.20	0.00
Maximum	174.40	188.50	155.80	336.30	353.80	618.70	567.00	419.10	399.90	342.70	209.00	87.70
Count	30.00	29.00	29.00	29.00	29.00	30.00	30.00	29.00	30.00	30.00	30.00	30.00
C/Lev.(95.0%)	12.22	14.84	15.42	30.50	25.21	41.87	54.65	35.28	34.27	23.70	18.16	10.62

Table 4: Descriptive statistics of the rainfall data for Ondo in coastal region

Ondo	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Mean	6.84	32.46	101.22	172.14	179.69	246.04	236.27	165.80	274.46	176.45	45.46	10.60
Std. Dev.	10.82	34.69	54.08	89.98	65.21	64.58	113.91	105.37	96.71	61.00	35.93	18.78
Samp. Var.	117.00	1203.74	2924.44	8097.15	4252.62	4170.91	12975.94	11103.68	9353.74	3720.76	1290.81	352.69
Kurtosis	2.29	0.70	-0.37	6.50	4.31	0.96	0.92	0.74	2.22	0.48	1.91	2.48
Skewness	1.78	1.22	0.00	2.05	1.51	0.89	0.86	0.90	1.03	0.45	1.25	1.85
Range	38.80	125.40	212.80	471.70	324.10	266.70	491.20	424.80	458.20	265.90	149.30	65.20
Minimum	0.00	0.00	0.00	41.60	86.60	140.80	61.00	30.10	98.40	66.40	0.00	0.00
Maximum	38.80	125.40	212.80	513.30	410.70	407.50	552.20	454.90	556.60	332.30	149.30	65.20
Count	29.00	30.00	29.00	29.00	29.00	28.00	27.00	27.00	27.00	26.00	26.00	28.00
C/Lev.(95.0%)	4.11	12.96	20.57	34.23	24.81	25.04	45.06	41.68	38.26	24.64	14.51	7.28

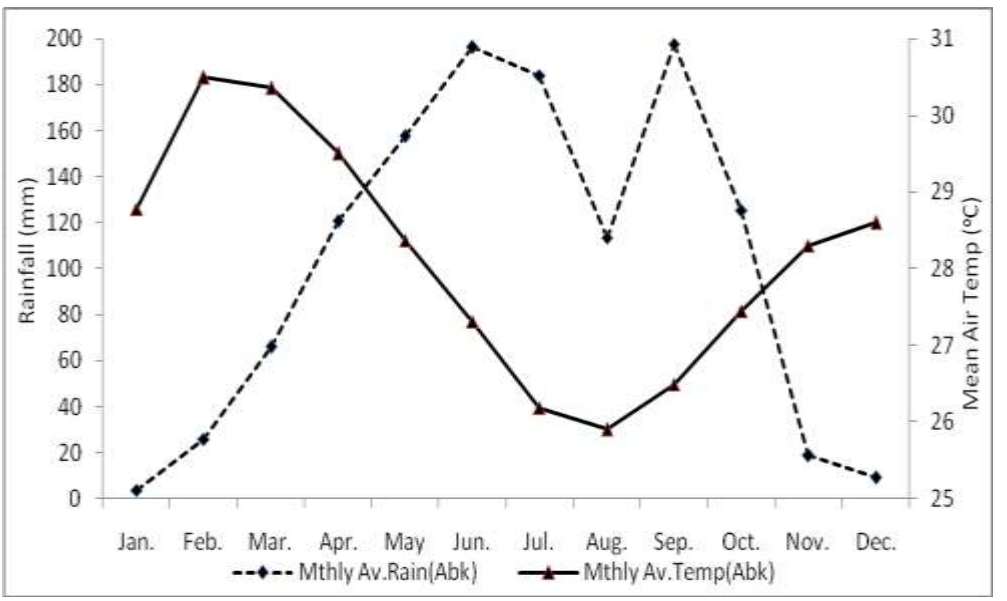

Figure 1: The monthly variations of rainfall and temperature at Abeokuta station

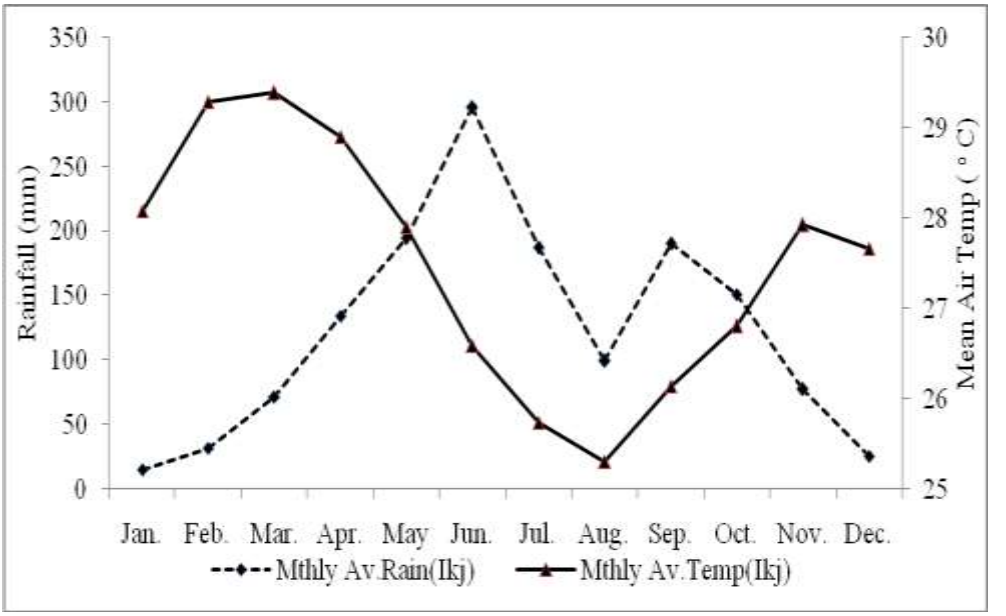

Figure 2: The monthly variations of rainfall and temperature at Ibadan station

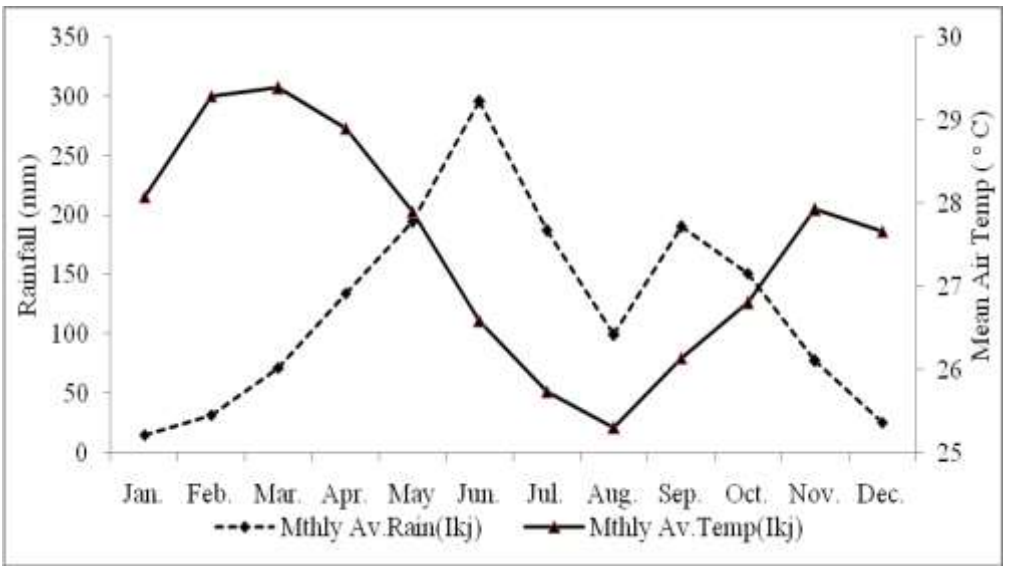

Figure 3: The monthly variations of rainfall and temperature at Ikeja station

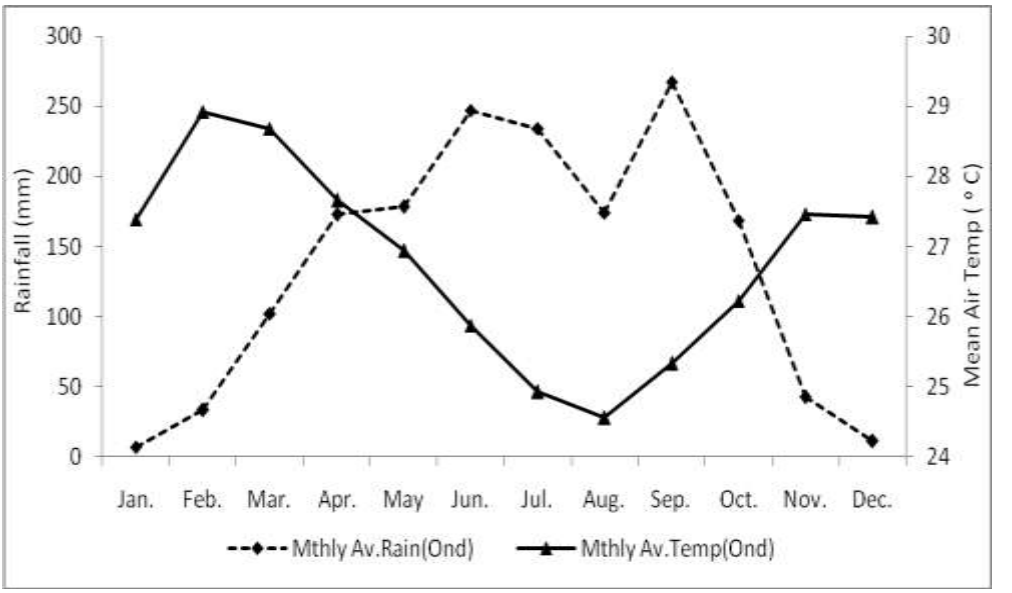

Figure 4: The monthly variations of rainfall and temperature at Ondo station

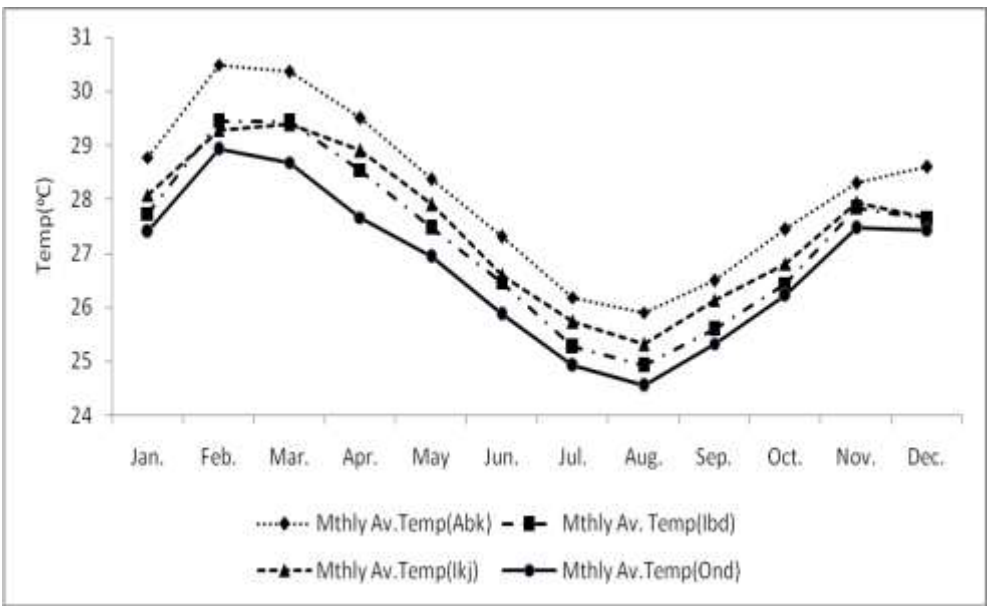

Figure 5: Monthly average temperature for Abeokuta, Ibadan, Ikeja and Ondo

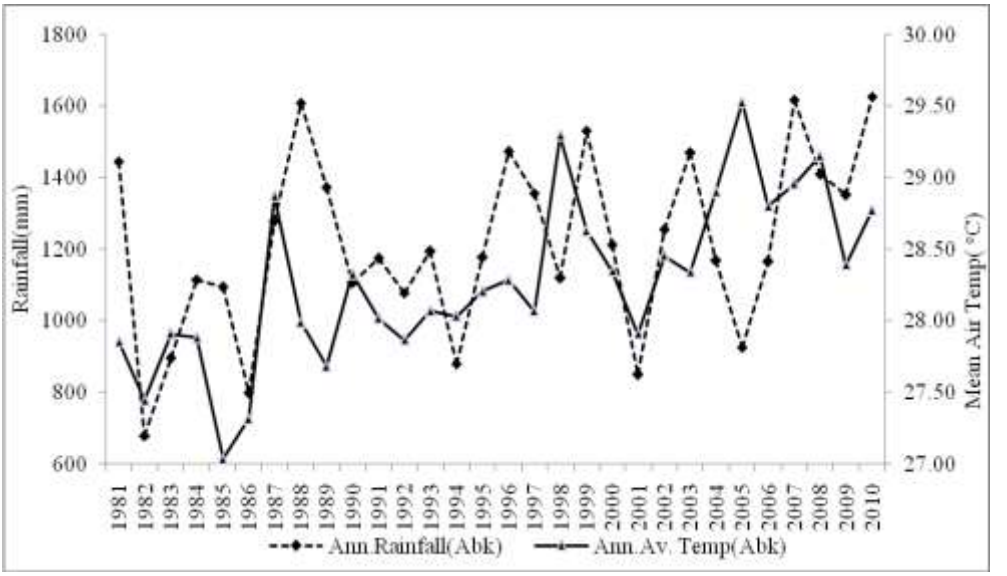

Figure 6: The annual variations of rainfall and temperature at Abeokuta station

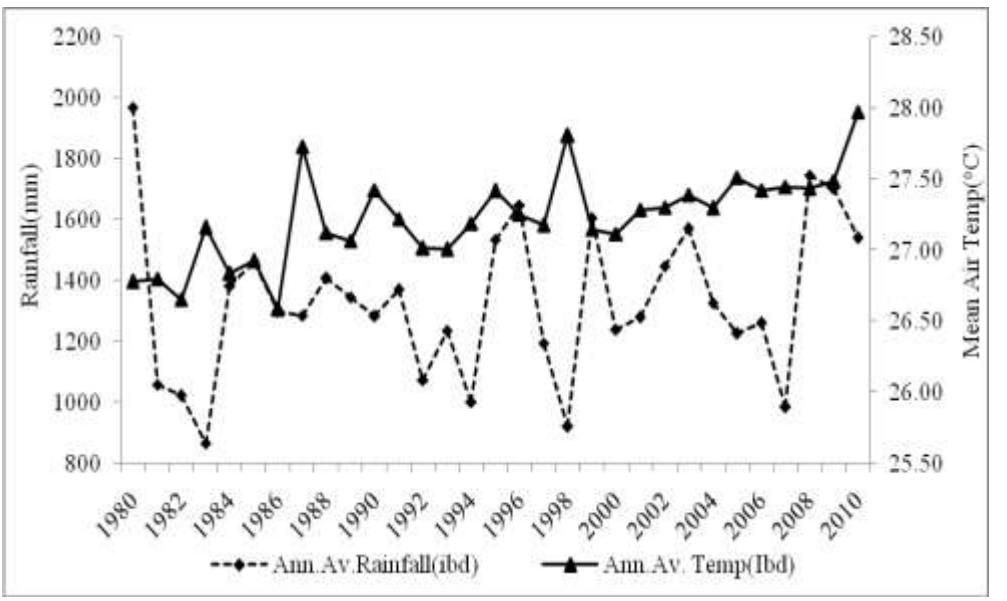

Figure 7: The annual variations of rainfall and temperature at Ibadan station

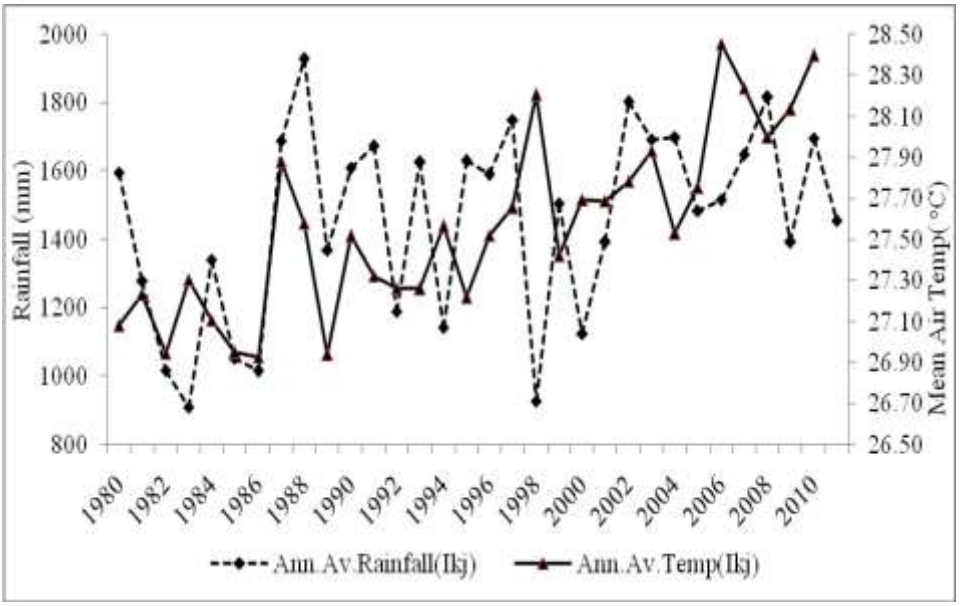

Figure 8: The annual variations of rainfall and temperature at Ikeja station

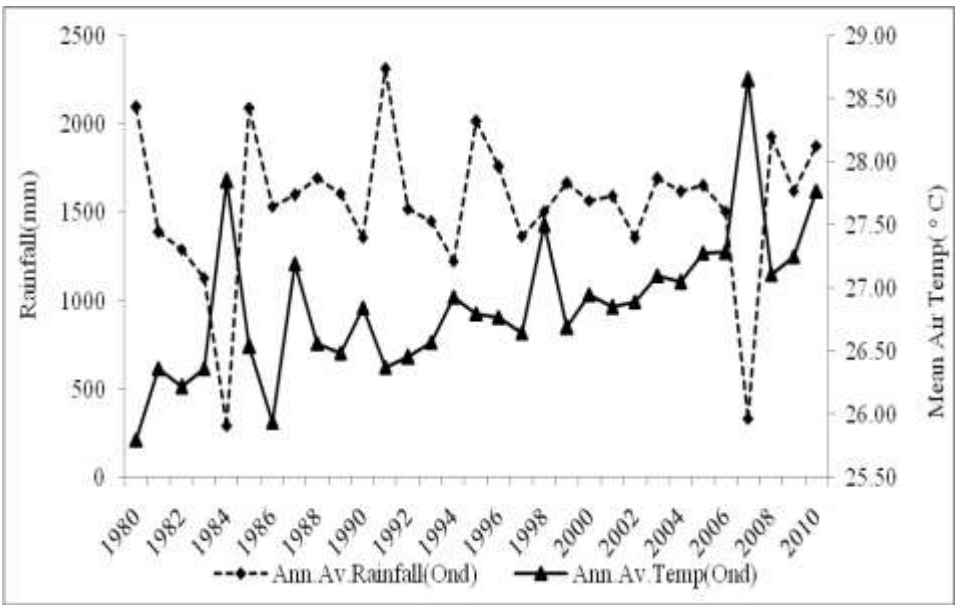

Figure 9: The annual variations of rainfall and temperature at Ondo station
Trend line equations for annual total rainfall (T_R) t = time in year

Trend line equations for annual total rainfall (T_R) t = time in year

$T_R = 10.76t + 1046.20$ (Abeokuta) ……(1)

$T_R = 5.25t + 1247.80$ (Ibadan) ……(2)

$T_R = 11.32t + 1267.90$ (Ikeja) ……(3)

$T_R = 2.23t + 1500.70$ (Ondo) ……(4)

Trend line equations for annual mean temperature (θ) t = time in year

$\theta = 0.047t + 27.54$ (Abeokuta) …….(5)

$\theta = 0.024t + 26.83$ (Ibadan) ……(6)

$\theta = 0.039t + 26.94$ (Ikeja) ……(7)

$\theta = 0.041t + 26.22$ (Ondo) ……(8)

Tables 1 to 4 showed the descriptive statistics of the four meteorological stations on monthly basis. Figures 1 to 4 showed the monthly variations of rainfall and temperature of stations under consideration, while Figure 5 showed the monthly variation of mean temperature of all the stations combined. The annual variations of rainfall and temperature for Abeokuta, Ibadan, Ikeja and Ondo stations were respectively shown in Figures 6, 7, 8 and 9.

(a) Monthly average and annual total rainfall trend

Generally, there was no month of the year that recorded zero as mean rainfall in all the stations considered. As far as monthly distribution of rainfall is concerned, a fairly "M-shaped" pattern of rainfall with bi-modal distribution was observed in the four stations under consideration. Two distinct peaks occurred in June and September with a little break/reduction of rainfall in August between the peaks. The primary and highest peak of rainfall was recorded in June in the coastal region of Ikeja. The primary peaks of other stations occurred in September. The "August break" may be due to the African-easterly Jet and over lain of the area by the Continental Tropical air mass as it was observed by other investigators [11-12]. Analyses of annual trends over a long period revealed a sequence of alternately decreasing and increasing trends in mean annual rainfall and air temperature in south western Nigeria during the study period. Generally over the years, there was an increasing trend in rainfall in all the stations under consideration in the coastal and derived savannah regions of southwestern of Nigeria. The gradients of the trend lines equations (Equations (1) to (4)) revealed the average annual increase in temperature as 10.76mmyr^{-1}, 5.25mm yr^{-1} , 11.32mm yr^{-1} and 2.23mm yr^{-1} for Abeokuta, Ibadan, Ikeja and Ondo respectively.

Abeokuta

Table1 showed that, zero value of minimum rainfall was recorded in January, February, November and December. Abeokuta recorded highest mean value of rainfall of 197.59mm in September with maximum value of rainfall of 436.30mm in June. Highly skewed rainfall was recorded in seven months (January, March, April, May, June, November and December) ; while rainfall was approximately symmetric in five months (February, July, August, September, and October). Kurtosis analyses showed that the distribution of rainfall in ten months (February to November) in Abeokuta were platykurtic, two months (January and December) were leptokurtic.

The average annual total rainfall over the period of consideration was for Abeokuta was 1213.1 mm. The maximum annual total rainfall of 1625.4mm occurred in the year 2010 while the minimum annual total rainfall of 677.1mm occurred in the year 1982. Generally over the years, there is an increasing trend in annual total rainfall. The rainfall distribution over the years was highly skewed while Kurtosis analyses showed that the distribution is platykurtic.

Ibadan

Ibadan recorded zero value of minimum rainfall in five months, namely, January, February, March, November and December. Ibadan recorded highest mean value of rainfall of 193.30mm in September with maximum value of rainfall of 411.30mm in August. Highly skewed rainfall was recorded in Ibadan in four months, namely: January, February, November and December. August and September recorded moderately skewed rainfall while approximately symmetric rainfall was recorded in six months (March, April, May, June, July and October). Kurtosis analyses showed that the distribution of rainfall in January in Ibadan was mesokurtic, nine months (February to October) were platykurtic and two months (November and December) were leptokurtic.

The average annual total rainfall over the period of consideration was for Ibadan was 1331.8 mm. The maximum annual total rainfall of 1967.7mm occurred in the year 1980 while the minimum annual total rainfall of 677.1mm occurred in the year 1982. Generally over the years, there is an increasing trend in annual total rainfall. The skewness of the annual total rainfall distribution was 0.3031, implying that the distribution is approximately symmetric. While the Kurtosis analyses of 0.00125 revealed that the distribution was platykurtic.

Ikeja

Ikejarecorded zero value of minimum rainfall in January, February and December. Highest mean value of rainfall of 296.58mm was recorded in June with maximum value of rainfall of 618.70mm also in June. The skewness of rainfall was high in Ikeja during the following six months: January, February, April, August and October. Rainfall in June, September, November and December were moderately skewed. While in March and May, the rainfall was approximately symmetric. Kurtosis analyses showed that the distribution of rainfall in nine months (March, April, May, June, July, September, October November and December) were platykurtic and three months (January, February and August) were leptokurtic.

The average annual total rainfall over the period of consideration was for Ikeja was 1454.7 mm. The maximum annual total rainfall of 1927mm occurred in the year 1988 while the minimum annual total rainfall of 909.1mm occurred in the year 1983. Generally over the years, there is an increasing trend in annual total rainfall. The skewness of the annual total rainfall distribution was -0.491, implying that the distribution is approximately symmetric. While the Kurtosis analyses of -0.893 revealed that the distribution was platykurtic.

Ondo

Like Ikeja, Ondo equally recorded zero value of minimum rainfall in January, February and December. Highest mean value of rainfall of 274.46mm was recorded in September with maximum value of rainfall of 556.60mm also in September. The skewness of rainfall was high in Ondo during the following

seven months: January, February, April, May, September, November and December. Rainfall in June, July and August were moderately skewed. While in March and October, the rainfall was approximately symmetric. Kurtosis analyses showed that the distribution of rainfall in ten months (January, February, March, June, July, August, September, October
November and December) were platykurtic and two months (April and May) were leptokurtic. Ondo recorded an average annual total rainfall of 1536.4mm over the period of consideration. The maximum annual total rainfall of 2310.2mm occurred in the year 1991 while the minimum annual total rainfall of 293.8mm occurred in the year 1984. Generally over the years, there is an increasing trend in annual total rainfall. The skewness of the annual total rainfall distribution was -1.31, implying that the distribution is approximately symmetric. While the Kurtosis analyses of 3.38 revealed that the distribution was highly leptokurtic.

(b) Monthly average and annual average temperature trend

Generally, the monthly average temperature in all the stations considered followed the same shape of oscillating pattern with the maximum mean temperature occurring in February-March and the minimum mean temperature occurring in August. Figure 5 revealed that the mean temperature each month generally increase in the order: Ondo, Ibadan, Ikeja and Abeokuta; with respective monthly mean values of 26.78^{o}C, 27.23^{o}C, 27.48^{o}C and 28.14^{o}C. The
corresponding altitude of the stations are: 287m (Ondo), 227m (Ibadan), 39m (Ikeja) and 67m (Abeokuta).The order of trend in monthly mean temperature of these stations is attributed to the combined effect of altitudes, in "that the higher we go, the cooler it is" coupled with the nearness of Ikeja to the sea. There is a negative correlation between the monthly mean temperature and monthly mean rainfall, that is, as the amount of rainfall increased, the temperature decreased. The correlation coefficients between the monthly mean temperature and monthly mean rainfall were -0.716, -0.673, -0.504 and -0.621 respectively for Ondo, Ibadan, Ikeja and Abeokuta respectively. For January, February and March the monthly mean temperature distributions were moderately skewed. In case of April, September to December, the distributions were highly skewed. While for May to August, the distributions were approximately symmetric. Kurtosis analyses showed that the distribution of mean temperature in nine months (January to August, and November) were platykurtic and three months (September, October and December) were leptokurtic. Generally over the years, there was an increasing trend in temperature in all the stations under consideration in the coastal and derived savannah regions of southwestern of Nigeria. The gradients of the trend lines equations (Equations (5) to (8)) revealed the average annual increase in temperature as 0.0474 ^{o}Cyr^{-1}, 0.0244^{o}C yr^{-1} , 0.039 ^{o}C yr^{-1} and 0.0406^{o}C yr^{-1} for Abeokuta, Ibadan, Ikeja and Ondo respectively.

The average annual mean temperature for Abeokuta was 28.27^{o}C. The maximum annual mean temperature of 29.53^{o}C occurred in the year 2005 while the minimum annual mean temperature

of 27^{o}C occurred in the year 1985. Kurtosis analyses of the average annual mean temperature distribution was platykurtic, while the skewness of the distribution was approximately symmetric.

The analysis revealed that the average annual mean temperature for Ibadan was 27.22^{o}C. The maximum annual mean temperature of 27.97^{o}C occurred in the year 2010 while the minimum annual mean temperature of 26.58^{o}C occurred in the year 1986. Kurtosis analyses of the average annual mean temperature distribution was platykurtic, while the skewness of the distribution was approximately symmetric.

The average annual mean temperature for Ikeja was 27.56^{o}C. The maximum annual mean temperature of 28.45^{o}C occurred in the year 2006 while the minimum annual mean temperature of 26.93^{o}C occurred in the year 1986. Kurtosis analyses of the average annual mean temperature distribution was platykurtic, while the skewness of the distribution was approximately symmetric.

The average annual mean temperature for Ondo was 26.87^{o}C. The maximum annual mean temperature of 28.65 ^{o}C occurred in the year 2007 while the minimum annual mean temperature of 25.8^{o}C occurred in the year 1980. Kurtosis analyses of the average annual mean temperature distribution was platykurtic, while the distribution was moderately skewed.

Conclusion

The analysis of rainfall and temperature over four meteorological stations in southwest Nigeria, falling into the coastal and derived savannah climatic regions has been studied extensively. It was observed that the monthly rainfall pattern followed a bi-modal distribution with three stations (Abeokuta, Ibadan and Ondo) having primary peak in September while that of Ikeja occurred in June. The secondary peak for Ikeja was experienced in September with others recording theirs in June."August break" was attributed to the Africa Eastern Jets and Tropical continental air mass of the West African Monsoon.

There was generally an increasing trend in rainfall amounts and frequency from January to June and decreasing trend from September to December in all the stations. Generally, the monthly average temperature in all the stations considered followed the same shape of oscillating pattern with the maximum mean temperature occurring in February-March and the minimum mean temperature occurring in August. The monthly mean temperature was discovered to be a function of altitude of the station as well as the nearness to the coast.

There was an increasing trend in temperature in all the stations for the period under consideration. Having studied and analyzed the rainfall and temperature pattern of southwestern Nigeria for about three decades, the increasing trends of both rainfall and temperature is an indicator that this region is having its fair share in the global climate change.

Acknowledgement
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References

1.Intergovernmental Panel on Climate Change (IPCC) (2007), Climate change 2007: climate change impacts, adaptation and vulnerability. *Working Group II; contribution to the Intergovernmental Panel on Climate Change Fourth Assessment Report. Summary for Policymakers,* 23

2.Eludoyin, O. (2009). Monthly variation in the 1985-1994 and 1995-2004 rainfall distribution over five selected synoptic stations in Western Nigeria. *Nigeria Journal of Meteorology and Climate Science,* 7:11–22

3.Ayansina, A., Ogunbo, S. (2009). GIS approach in assessing seasonal rainfall variability in Guinea savanna part of Nigeria. *7th FIG Regional Conference, Vietnam,*16

4.Hasanean, H. M. (2001). Fluctuations of surface air temperature in the east Mediterranean. *Theoret Appl Climatol*, 68(1–2):75–87

5.Turkes M, Sumer UM, Demir I (2002) Re-evaluation of trends and changes in mean, maximum and minimum temperatures of Turkey for the period 1929–1999. *Int J Climatol*, 22:947–977

6.Oriola, E. (1994). Strategies for combating urban flooding in a developing nation: a case study of Ondo, Nigeria. *The Environmentalist,* 4:57–62

7.Bunting, A. H., Dennett, M. D., Eliston, J., & Milford, J. R. (1976). Rainfall trends in the West Africa Sahel. *Q.J.R. Meteorological Society,* 102:56–64

8.Folland, C. K., Palmer, T. N., Parker, D. E. (1986). Sahel rainfall and worldwide sea temperatures 1901-85: observational, modelling and simulation studies. *Nature*, 320:602–607

9.Odekunle, T. (2001). The magnitude frequency characteristic of rainfall in Ondo, Southwestern, Nigeria. *Ife Research Publications in Geography*, 8:36–41

10.Ologunorisa, E. (2001). An assessment of flood risk in the Niger Delta, Nigeria. Ph.D Thesis, Department of Geography and Environmental Management, University of Port -Harcourt, Port Harcourt.

11.Ilesanmi, O. (1981). Aspect of the precipitation climatology of the July-August rainfall minimum of southern Nigeria. Urban and regional planning problems in Nigeria. *University of Ile-Ife Geographical Associations Regional Planning Committee*

12.Omotosho, J. (2007). Pre-rainy season moisture build-up and storm precipitation delivery in the West Africa Sahel. *International Journal of Climatology*, 28:937–946

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Listeria Species in Seafoods from two Major Fish Markets in Lagos, Nigeria

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Abstract: *Listeria* is a bacterial genus thatis widely distributed in our environment. Its most economically important species is *Listeria monocytogenes*. Listeriosis is a serious infection caused by eating food contaminated with *Listeria monocytogenes*. The contamination of seafoods with *Listeria* species may occur during processing, handling and packaging due to poor quality control measures. The aim of this study was to isolate and identify *Listeria* spp. from seafoods sold at Liverpool and Makoko fish markets in Lagos. A total of 193 seafood samples including Blue Whiting (*Micromesistus poutasou*), Croaker (*Pseudotolitus elongatus*) and Pink Shrimp (*Penaeus notialis*) were screened for the presence of *Listeria* spp. The isolation and identification of *Listeria* species were carried out using the Oxoid *Listeria* Précis method. Forty-nine samples were positive for *Listeria* spp. and were identified as *Listeria ivanovii*,*L. grayi*, *L. welshimeri*, *L. monocytogenes* and *L. innocua*. Fresh croaker had the highest prevalence of 58.8%. The results of this study indicate the presence of *Listeria* spp. in seafoods in Liverpool and Makoko fish markets. It also revealed the possibility that these seafoods could contribute to food borne infections. Therefore, the improvement of seafood quality is of utmost importance.

Keywords: Seafoods, *Listeria* species, contamination, fish markets.

Introduction

Listeria is a bacterial genus thatis widely distributed in our environment. They are motile Gram-positive short rods, non-spore formers, catalase positive and oxidase negative [1]. The genus *Listeria* currently consist of 17

recognized species: *Listeria monocytogenes, L. seeligeri, L. ivanovii, L. welshimeri, L.marthii, L. innocua, L. grayi, L. fleischmannii, L. floridensis, L. aquatica, L.newyorkensis, L. cornellensis, L. rocourtiae, L. weihenstephanensis, L. grandensis, L. riparia* and *L. booriae*. Of all these, *L. monocytogenes* and *L. ivanovii* are considered pathogens [2]. *L. monocytogenes* is of major concern for public health authorities and the food industry, as the cold-tolerant organism is known to cause human infections and has been associated with a large number of foodborne disease outbreaks [3-6]. It is endowed with numerous adaptive physiological traits that enable it to survive under a wide range of environmental conditions [7]. Implicated foods include milk products, vegetables, salads, seafoods (especially ready-to-eat seafoods) and meat products [8]. Listeriosis is a serious infection caused by eating food contaminated with *Listeria monocytogenes*. The disease affects primarily pregnant women, newborns and adults with weakened immune systems [9]. It has mortality rate of 20-30% and hospitalization rate of 91% [10]. Consumers' awareness of nutrition and food quality has led to the increased consumption of seafood products. Seafood is recommended by nutritionists because of its high nutritional value [11]. However, along with the nutrients and benefits gotten from seafood consumption come the potential risks of eating contaminated seafood [12]. Seafood are susceptible to several food poisoning organisms as well as to some that are unique to marine products such as *Clostridium botulinum, Yersinia enterocolitica*, *Listeria monocytogenes* and *Vibrio parahaemolyticus* [13]. The occurrence of *Listeria* species in seafoods have been investigated in several countries but little has been reported about it in Nigeria. The contamination of seafoods with *Listeria* species may occur during processing, handling and packaging due to poor quality control measures. The aim of this study was to isolate and identify *Listeria* spp. from seafoods sold at Liverpool and Makoko fish markets in Lagos State in order to generate information on the prevalence of this pathogen so as to provide baseline information for Nigerian regulatory authorities to allow the formulation of a regulatory framework for controlling *Listeria* spp. and ensuring seafood safety.

Materials and Methods

Sample Collection

A total of 193 seafood samples including Blue Whiting (*Micromesistus poutasou*), Croaker (*Pseudotolitus elongatus*) and Pink Shrimp (*Penaeus notialis*) were screened for the presence of *Listeria* spp. The samples were obtained monthly for a year from two fish markets (Liverpool and Makoko) in Lagos State.

Isolation and identification of *Listeria* species

The isolation and identification of *Listeria* species was carried out using the Oxoid Listeria Précis method. The identification of *Listeria* was carried out according to the methods described in the Bacteriological Analytical Manual [14]. Some rapid methods such as Oxoid Biochemical Identification System (O.B.I.S.), Oxoid Listeria Test Kit and MICROBACT Listeria 12L system were also used for identification of *Listeria* isolates.

Statistical analysis

A test of significance of the prevalence of *Listeria* spp. in seafoodwas carried out using the one sample t-test computed using SPSS package. A difference of 95% was used in the analysis, $p<0.05$ level was considered to be statistically significant.

Results

Phenotypic characterisation of *Listeria* isolates showed that all isolates were Gram positive short rods, catalase positive and oxidase negative. They were all motile and tested positive for *Listeria* latex agglutination test. The species of *Listeria* identified by Microbact *Listeria* 12L system were *Listeria ivanovii, L. grayi, L. welshimeri, L. monocytogenes* and *L. innocua*. Furthermore,of the 193 seafood samples, 49 (25.4%) were found positive for *Listeria* spp. which were identified as *Listeria ivanovii* (16, 8.3%), *Listeria welshimeri* (12, 6.2%), *Listeria monocytogenes* (12, 6.2%), *Listeria grayi* (5, 2.6%) and *Listeria innocua* (4, 2.1%). Fresh croaker had the highest prevalence of 58.8%. Species wise, 16 isolates of *L. ivanovii* were all from fresh croaker; 12 *L. welshimeri* isolates included six recovered from fresh shrimp, four from smoked blue whiting and two from fresh croaker; 12 *L. monocytogenes* comprised of eight isolates from fresh croaker, three from smoked blue whiting and one from smoked shrimp; 5 *L. grayi* isolates that consisted of three smoked shrimp and two smoked blue whiting and 4 isolates of *L. innocua* recovered from fresh croaker (Table1). *Listeria* species were not isolated from smoked croaker and frozen blue whiting. In Liverpool market, *L. monocytogenes* were recovered from fresh croaker (20%), smoked blue whiting (12%) and smoked shrimp (10%) (Table 2). *L. welshimeri* was isolated from fresh croaker (9.5%), smoked blue whiting (13.3) and fresh shrimp (20%) in Makoko market (Table 3). The statistical analysis conducted showed that the prevalence of *Listeria* spp. in seafoods was significant at 95% confidence limit ($p<0.05$).

Table 1: Prevalence of *Listeria* species in seafoods

Seafood Samples	Number of Samples Analyzed	*Listeria* spp. positive, n (%)	*L. grayi* n (%)	*L. innocua* n (%)	*L. ivanovii* n (%)	*L. welshimeri* n (%)	*L. monocytogenes* n (%)	
Fresh Croaker	51	30 (58.8)	0 (0.0)	4 (7.8)	16(31.4)	2 (3.9)	8 (15.7)	
Smoked Croaker	41	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Frozen Blue Whiting	3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Smoked Blue Whiting	55	9 (16.4)	2 (3.6)	0 (0.0)	0 (0.0)	4 (7.3)	3(5.5)	
Fresh Shrimp	24	6 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (25.0)	0 (0.0)	
Smoked Shrimp	19	4 (21.0)	3 (15.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)	0 (0.0)
Total	193	49 (25.4)	5 (2.6)	4 (2.1)	16 (8.3)	12(6.2)	12(6.2)	

Key: n: number of positive samples

Table 2: Prevalence of *Listeria* species in seafoods from Liverpool market

Seafood Samples	Number of Samples Analyzed	*Listeria* spp. positive, n (%)	*L. grayi* n (%)	*L. innocua* n (%)	*L. ivanovii* n (%)	*L. welshimeri* n (%)	*L. monocytogenes* n (%)	
Fresh Croaker	30	18 (60.0)	0 (0.0)	2 (6.7)	10 (33.3)	0 (0.0)	6 (20.0)	
Smoked Croaker	24	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Frozen Blue Whiting	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Smoked Blue Whiting	25	3 (12.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (12.0)	
Fresh Shrimp	9	3 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (33.3)	0 (0.0)	
Smoked Shrimp	10	2 (20.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)
Total	98	26 (26.5)	1(1.0)	2(2.0)	10 (10.2)	3 (3.1)	10 (10.2)	

Key: n: number of positive samples

Table 3: Prevalence of *Listeria* species in seafoods from Makoko market

Seafood Samples	Number of Samples Analyzed	*Listeria* spp. positive, n (%)	*L. grayi* n (%)	*L. innocua* n (%)	*L. ivanovii* n (%)	*L. welshimeri* n (%)	*L. monocytogenes* n (%)	
Fresh Croaker	21	12 (57.1)	0 (0.0)	2 (9.5)	6 (28.6)	2 (9.5)	2 (9.5)	
Smoked Croaker	17	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Frozen Blue Whiting	3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Smoked Blue Whiting	30	6 (20.0)	2 (6.7)	0 (0.0)	0 (0.0)	2 (6.7)	2(6.6)	
Fresh Shrimp	15	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (20.0)	0 (0.0)	
Smoked Shrimp	9	2 (22.2)	2 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	95	23 (24.2)	4 (4.2)	2 (2.1)	6 (6.3)	7 (7.4)	4 (4.2)	

Key: n: number of positive samples

Discussion

The results from this study have shown that there is a significant difference in the prevalence of *Listeria* spp. in seafoods. Also, prevalence of *Listeria* spp. in seafoods from Liverpool and Makoko markets are significant with the exception of smoked shrimp from Liverpool market. The occurrence of *Listeria* spp. isolated from seafood as shown in Table1 is in accordance with earlier reports in Nigeria and other countries [15-19]. *Listeria ivanovii, L. grayi, L. welshimeri, L. monocytogenes* and *L. innocua* were isolated from Liverpool and Makoko markets which are primary fish markets in Lagos (Tables 2&3). This is similar to the results of Modaresi *et al*. [16] who also isolated these five *Listeria* spp. from seven fish species in Urmia fish markets in Iran. The results in this study has shown that severe controls need to be undertaken on the hygienic quality of these seafood so as not to lead to an outbreak since these markets are major fish markets. Furthermore, food safety and quality standards need to be adhered to in order to control the growth of *Listeria* during fishing, collection, transmission, distribution and storage [20]. Liverpool fish market in Lagos State, Nigeria, plays a prominent role in

the distribution of fishery products across the state.

The market lacks social amenities such as water and sanitary facilities. The only available water used was taken from the Lagoon [21].The same scenario also occurs in Makoko fish market. The coexistence of several *Listeria* species on the same food is not unusual and often the prevalence of *Listeria* species is higher than that of *L. monocytogenes* [22]. The differences in prevalence of *Listeria* spp. in seafood might be attributed to the type of seafood, source of samples, number of samples, method of sampling, sampling season, isolation method, human activity, geographical area, climate of area and sensitivity of bacteriological detection methods [18,20,23]. Also, the presence of non-pathogenic species such as *L. innocua* may indicate potential contamination with *L. monocytogenes* [24]. Apart from *Listeria monocytogenes*, other *Listeria* spp. such as *L. ivanovii, L. seeligeri, L. innocua, L. welshimeri* are regarded as non-pathogenic to human, and have been implicated in human infections. Also, it is believed that the occurrence of non-invasive listeriosisis underestimated because *L. monocytogenes* is not among the pathogens routinely investigated in the outbreaks of gastro-intestinal diseases [25,26].

The results of the current study demonstrated that 25.4% of seafood samples tested was contaminated with *Listeria* spp. and 6.2% were contaminated with *L. monocytogenes*. Contamination with *L. monocytogenes* may occur long before the raw material reaches retail trade or processing factories. The main sources of *L. monocytogenes* are contamination from water and ice, soiled surfaces and boxes, as well as contamination from human and avian sources [27]. Not much is known about the potential *Listeria* contamination of fish and fish products at the retail level. Products that are purchased in large quantities and re-packaged prior to sale may be at risk to *L. monocytogenes* contamination. Despite the occurrence of *L. monocytogenes* in raw and frozen seafood, these products do not pose a threat to the majority of people as they undergo some processing before being eaten. However, they still pose risk to susceptible populations when consumed raw or lightly cooked [27].The occurrence of *Listeria* spp. in cooked Ready-To-Eat seafood samples could be from the cross-contamination during fish handling, incomplete cleaning and disinfection procedures, and incomplete implementation of HACCP principles in processing plants.

Conclusion

The results of this study indicate the presence of *Listeria* spp. in seafoods in Liverpool and Makoko fish markets. The presence of *Listeria* spp. particularly *L. monocytogenes* in smoked and uncooked products could be a potential risk for consumers. Also, the findings in this study has provided basic information on the occurrence of *Listeria* spp. in seafoods sold at Liverpool and Makoko fish markets in Lagos State. This information can be used by the Nigerian food safety authorities to formulate a regulatory framework for controlling *Listeria* spp. and ensuring seafood safety.

References

1.Seeliger, H.P.R., Jones, D. (1984). Genus Listeria Pirie, 1940, 383AL p. 1235-1245. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's Manual of Systematic Bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.

2.Orsi, R.H., Wiedmann, M. (2016). Characteristics and distribution of Listeria spp., including Listeria species newly described since 2009. *Applied Microbiology and Biotechnology*, 100: 5273-5287.

3.Farber, J.M., Peterkin, P.I. (1999). *Listeria monocytogenes*,a Food-Borne Pathogen. *Microbiological Reviews*, 55(3): 476-511.

4.Jacquet, C., Catimel, B., Brosch, R., Buchriese, C., Dehaumont, P., Goulet, V. *et al*., (1995). Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. *Applied and Environmental Microbiology*, 61: 2242-2246.

5.Dalton, C.B., Austin, C.C., Sobel, J., Hayes, P.S. Bibb, W.F., Graves, L.M., *et al*., (1997). An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. *New England Journal of Medicine*, 336:100-105.

6.Miettinem, M., Bjorkroth, J., Korkeala, H. (1999). Characterization of *Listeria monocytogenes* from an ice cream plant by serotyping and pulsed-field gel electrophoresis. *International Journal of Food Microbiology*, 46:187-192.

7.Borucki, M.K., Peppin, J.D., White, D., Loge, F., Call, D.R. (2003). Variation in biofilm formation among strains of *Listeria monocytogenes*. *Applied and Environmental Microbiology*, 69: 7336-7342.

8.Ben Embarek, P.K. (1994). Presence, detection and growth of *Listeria monocytogenes* in seafoods: A Review. *International Journal of Food Microbiology*, 23:17-34.

9.Todar, K. (2008). *Listeria monocytogenes*. Todar's Online Textbook of Bacteriology. www.textbookofbacteriology.net

10.Mead, P.S., Slutsker L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., *et al*., (1999).Food-related illness and death in the United States. *Emerging infectious Diseases*, 5: 607-625.

11.Ghanbari, M., Jami, M., Domig, K.J., Kneifel, W. (2013). Seafood biopreservation by lactic acid bacteria – a review. *LWT – Food Science and Technology*, 50(2):315–324.

12.Iwamoto, M., Ayers, T., Mahon, B.E., Swerdlow, D.L. (2010). Epidemiology of seafood associated infections in the United States. *Clinical Microbiology Reviews*, 23(2): 399-411.

13.Ali, F.H.M. (2011). Quality Evaluation of Some Fresh and Imported Frozen Seafood. *Advance Journal of Food Science and Technology*, 3(1): 83-88.

14.Hitchins, A.D., Jinneman, K. (2011). Detection and Enumeration of *Listeria monocytogenes* in Foods. Chapter 10. In: Bacteriological Analytical Manual. U.S. Food and Drug Administration, Silver Spring.

15.Salihu, M.D., Junaidu, A.U., Manga, S.B., Gulumbe, M.L., Magaji, A.A., Ahmed, A., *et al*., (2008).

Occurrence of *Listeria monocytogenes* in smoked fish in Sokoto, Nigeria. *African Journal of Biotechnology*, 7: 3082-3084.

16.Modaresi, R., Mardani, K., Tukmechi, A., Ownagh, A. (2011). Prevalence of *Listeria* spp. infish obtained from Urmia fish markets. *African Journal Microbiology Research*, 5:5398–5401.

17.Adebayo-Tayo, A.C., Odu, N.N., Michael, M.U., Okonko, I.O. (2012). Multi-Drug Resistant (MDR) Organisms isolated from Sea-foods in Uyo, South-Southern Nigeria. *Nature and Science*, 10(3): 61-70.

18.Garedew, L., Taddese, A., Biru, T., Nigatu, S., Kebede, E., Ejo, M., *et al*., (2015). Prevalence and antimicrobial susceptibility profileof listeria species from ready-to-eat foods ofanimal origin in Gondar Town, Ethiopia. *BMC Microbiology*, 15:100.

19.Jamali, H., Paydar, M., Ismail, S., Looi, C.Y., Wong, W.F., Radmehr, B., *et al*., (2015). Prevalence, antimicrobial susceptibility andvirulotyping of *Listeria* species and *Listeria monocytogenes* isolated from open-air fishmarkets.*BMC Microbiology*, 15:144.

20.Momtaz, H., Yadollahi, S. (2013). Molecular characterization of *Listeria monocytogenes* isolated from fresh seafood samples in Iran. *Diagnostic Pathology*,8:149.

21.Ayo-Olalusi,C.I., Anyanwu, P.E., Ayorinde, F., Aboyweyere, P.O. (2010).The Liverpool fish market in Lagos State, Nigeria. *African Journal of Agricultural Research*, 5(19): 2611-2616

22.Johnson, J.L., Doyle, M.P., Cassens, R.G. (1990). *Listeria monocytogenes* and other *Listeria* spp. in meat and meat products, a review. *Journal of Food Protection*, 53:81-91.

23.Soultos, N., Iossifidou, E., Tzikas, Z., Sergelidis, D., Lazou, Th., Drakopoulos, G., *et al*., (2014). Prevalence of *Listeria monocytogenes* in ready-to-eat seafood marketed in Thessaloniki (Northern Greece). *Veterinary World*,7(11): 1004-1009.

24.Gasanov, U., Hughes, D., Hansbro, M. P. (2005). Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes:* a review. *FEMS Microbiology Reviews*, 29: 851-875.

25.Franciosa, G., Tartaro, S., Wedell-Neergaard, C., Aureli, P. (2001). Characterization of Listeria monocytogenes strains involved in invasive and noninvasive listeriosis outbreaks by PCR-based fingerprinting techniques. *Applied and Environmental Microbiology*, 67:1793–1799.

26.Ramaswamy, V., Cresence, V.M., Rejitha, J.S., Lekshmi, M.U., Dharsana, K.S., Prasad, S.P., *et al*., (2007). Listeria – Review of epidemiology and pathogenesis. *Journal of Microbiololgy, Immunology and Infection*, 40:4-13.

27.Parihar, V. S., Barbuddhe, S. B., Danielsson-Tham, M. L., Tham, W. (2008). Isolation and characterisation of *Listeria* species from tropical seafoods. *Food Control*, 19: 566–569.