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Occurrence and antibiotic resistance profile of *Listeria monocytogenes* in salad vegetables and vegetable salads sold in Zaria, Nigeria

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Listeria monocytogenes **is a Gram positive facultative anaerobic microorganism that causes listeriosis in both animals and humans. The main route of infection has been identified to be by ingestion of food contaminated with the organism particularly those stored at refrigeration temperatures. This study was carried out to determine the occurrence and antimicrobial resistance profile of *L. monocytogenes* in salad vegetables and ready to eat vegetable salads sold in Zaria metropolis. A total of 355 samples were collected which included 250 salad vegetable samples and 105 samples of salad and coleslaw from food vending outlets within the metropolis. Confirmation of the isolates using conventional biochemical characterization showed that 14 (3.9%) of the total samples collected were positive for *L. monocytogenes* while 95.9% of the samples were positive for other *Listeria* spp. Distribution by sample type showed that 11 (4.7%) were positive for *L. monocytogenes* while 2 (4.4%) and 1 (1.7%) was observed in coleslaw and convention vegetable salads, respectively though the difference was not statistically significant (p value = 0.587). Cabbage and lettuce had the highest prevalence (8.5 and 6.2%, respectively) though this was not statistically significant (p value=0.517). Antibiotic susceptibility testing by disc diffusion method showed that 92.9% of the isolates were resistant to ampicillin while ciprofloxacin has the lowest resistance (14.3%). Antimicrobial resistance profile of the isolates revealed that they were all resistant to at least one antimicrobial agent. The study therefore shows that *L. monocytogenes* occur in salad vegetables and ready to eat salads sold in Zaria, Nigeria, therefore, adequate precaution should be taken in preparing these vegetables for both human and animal consumption. Also, the antimicrobial resistance pattern shown by the isolates is an indication that adequate measure need be taken to regulate drug use in both humans and animals in order to minimize the risk of increasing antimicrobial resistance.**

Key words: *Listeria monocytogenes*, antimicrobial resistance, anaerobic microorganism.

INTRODUCTION

Vegetable salad is a mixture of fresh vegetables and cream milk that provides a rich source of minerals and dietary fibre of low fat and calories to the consumer (Udo et al., 2009; Adeshina et al., 2012). They may be in the form of conventional salad prepared from a mixture of carrot, cabbage, cucumber, tomatoes, lettuce with the addition of pre-packaged salad cream and baked beans (Itohan et al., 2011).

The consumption rates of vegetables and vegetable salads have also greatly increased based on their proven medical and nutritional benefits (Oyenuga and Fetuga, 1985; Abdullahi and Abdulkareem, 2010; Asagbra and

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Oyewole, 2002; Udo et al., 2009; Adeshina et al., 2012). A report by the National Bureau of Statistic (NBS) on the consumption pattern in Nigeria for the year 2009/2010 shows that out of the over 24 billion Naira total expenditure, 64.68% was the total household expenditure on food. About 10% of the total expenditure on food was spent on vegetables which include tomatoes, cabbage, onions, lettuce, cucumber, salad cream, okro, garden eggs and other vegetables. This proportion was only second to the amount spent on tuber which was 14.6% (NBS, 2012).

The infectious nature of these vegetables results from contamination with pathogenic microorganisms which occurs during harvesting, through human handling, harvesting equipments, transport containers, wild and domestic animals (Itohan et al., 2011). Washing fruits and vegetables in chlorinated water can reduce bacterial levels but cannot be relied upon to eliminate pathogens (Adebayo-Tayo et al., 2012).

Moreover, the availability of potable water for proper washing of these fruits and vegetables is also lacking in different areas. As a result of which dirty or contaminated water is used for washing which could lead to further increasing the microbial load on these vegetables which some people buy and eat without further washing. *Listeria monocytogenes* and *Salmonella* spp. have been isolated from raw vegetables making them potential threat to consumers (Dolye, 1990; Biniam and Mogessie, 2010) and some consumer eat these produce raw following purchase without any further washing.

Many countries in the world have developed policies that range from zero tolerance to tolerable limits depending on the food item. It is believed that occurrence of outbreaks is likely to have greater impact on the society later due to increasing number of susceptible population including elderly people and immuno-compromised persons including those with HIV/AIDS as well as an increasing consumption of ready-to-eat, refrigerated and extended shell-life foods (Pagotto et al., 2006).

The organism has been isolated in faecal samples of cattle, sheep, goats, chicken and pigs (Umeh and Okpokwasili, 2009; Chukwu, 2010) and water samples obtained from lakes in Nigeria (Nwachukwu et al., 2010a). Faeces from these animals and water from these lakes serve as a major source of fertilizer and water supply for the production of these vegetables.

The changes in life style have also resulted into more food been eaten away from home which can be evident by the increasing number of food vending outlets. The hygiene practices involved in processing these vegetables for human consumption still remains an issue of concern (Manchester and Clauson, 1995).

Reports on the outbreaks of *L. monocytogenes* infections in Nigeria is grossly inadequate because the disease produces febrile gastroenteritis in apparently healthy persons and has a course that is similar to gastro-enteritis caused by other organisms like *Salmonella* and *Campylobacter* spp. (FAO/WHO, 2004) which often makes diagnosis more obscure. This study therefore focuses on providing information on the quality of salad vegetables and ready-to-eat (RTE) Vegetable salads sold in Zaria with respect to *L. monocytogenes*.

MATERIALS AND METHODS

Two hundred and fifty (250) vegetables samples were collected from five daily markets in Zaria metropolis, as well as 59 vegetable salads and 46 coleslaw samples collected from food vending outlets as within the metropolis. These samples were bought and packaged as they are sold to other consumers. They were labeled appropriately and transported to the Bacterial Zoonosis Laboratory in the Department of Veterinary Public Health, Ahmadu Bello University, Zaria, Nigeria for analysis.

Isolation and identification of *L. monocytogenes*

The ISO 11920-1 (1996) method for qualitative isolation and identification of *L. monocytogenes* was used as described by Indrawattana et al. (2011) with modifications. Ten grams of each sample was placed into a stomacher bag containing 90 ml of Half Fraser broth (Oxoid, Basingstoke, UK). Food homogenization was done using a Stomacher (Stomacher Lab Blender 400) for 2 to 3 min, followed by incubation at 25°C for 24 h. Then, 0.1 ml of each sample were inoculated into 10 ml of Fraser's broth (FB) in a culture tube and incubated at 37°C for 48 h. A loop-full of each of positive FB culture, that is, dark colour caused by aesculin hydrolysis, was plated individually on Oxford *Listeria* agar (Oxoid, Basingstoke, UK), and the plates were incubated at 37°C for 24-48 h. The colonies suspected to be *L. monocytogenes* through Gram staining and catalase were stored on nutrient slant for further processing.

The isolates were further tested for haemolysis on blood agar and carbohydrate fermentation using mannitol, rhamnose and xylose as described by Janzten et al. (2006), and Jemmi and Stephan (2006). Organism that fermented mannitol and xylose, and positive to rhamnose were considered *L. monocytogenes*.

Antibiotic sensitivity

The antibiotic susceptibility of the isolates was determined by the disk diffusion method on Mueller-Hinton Agar (MHA, Oxoid, Basingstoke, UK). The antibiotics tested include: Sulfamethoxazole-Trimethoprim (25 µg), Amikacin (30 µg), Tetracycline (30 µg), Ampicillin (10 µg), Penicillin G (10 IU), Erythromycin (15 µg), Gentamicin (30 µg), Chloramphenicol (30 µg) Ciprofloxacin (5 µg), Streptomycin (10 µg), Oxacillin (5 µg) and Vancomycin (30 µg).

Three to five well isolated colonies of *L. monocytogenes* were transferred into 5 mL Brain Heart Infusion broth (BHIB, Oxoid) and incubated at 37°C for 18 to 24 h. The overnight broth culture was, diluted using sterile distilled water to a turbidity equivalent to 0.5 McFarland standard (approximately 10^8 cfu/ml), and inoculated onto the entire surface of a dried Mueller-Hinton Agar (MHA, Oxoid) plate creating a lawn of the culture. The inoculated MHA plates were allowed to dry at room temperature before placing the antibiotic discs followed by incubation. After incubation for 24 h at 37°C, the diameter (in mm) of the zone around each disk was measured and interpreted in accordance with the Clinical and Laboratory Standards Institute Standards guidelines (CLSI, 2011) using *Staphylococcus aureus* ATCC 25923 as control strain (Rodas-Suarez et al., 2006).

Table 1. Distribution of occurrence of isolates.

Sample type	Number collected	Number positive (%)	χ^2	p-value
Vegetables	250	11 (4.7)	1.066	0.587
Salad	59	1 (1.7)		
Coleslaw	46	2 (4.4)		
Total	340	14 (4.1)		

Table 2. Distribution of occurrence of isolates by vegetable type.

Sample type	Number collected	Number positive (%)	χ^2	p-value
Carrot	50	1 (2.2)	3.248	0.517
Cabbage	51	4 (8.5)		
Cucumber	51	1 (2.0)		
Lettuce	49	3 (6.2)		
Tomato	49	2 (4.3)		
Total	236	11 (4.7)		

RESULTS

Out of the 355 samples examined, 340 (95.8%) were positive for *Listeria* spp. while 15 (4.2%) showed no growth on *Listeria* agar, Oxford formulation. Among the positive samples, 14 (4.1%) were positive for *L. monocytogenes* following biochemical characterization. Distribution of the isolates by sample type showed that salad vegetables had the highest level contamination, 11 (4.7%) which was followed by the contamination level in coleslaw, 2 (4.4%) and the least contamination in conventional vegetable salad, 1 (1.7%) as shown in Table 1.

Listeria spp was isolated from 236 (94.4%) of the vegetables tested. Further biochemical analysis showed that 11 (4.4%) of the vegetables were positive for *L. monocytogenes*. Distribution by vegetable type showed that carrot and cucumber had the least contamination level each, 1 (2.2%) while cabbage had the highest contamination level, 4 (8.5%) followed by lettuce, 3 (6.2%) and tomatoes, 2 (4.3%) as shown in Table 2.

The antibiotic sensitivity test showed a high resistance to ampicillin (92.9%) followed by oxacillin (85.7%). The least resistance was obtained for ciprofloxacin (14.3%) and gentamicin (21.4%). The antimicrobial resistance profile showed that all the isolates were resistant to at least one antimicrobial agent though majority (64.3%) of the isolates were resistant to more than four antimicrobial agents (Figure 1 and Table 3).

DISCUSSION

L. monocytogenes was recovered from 4.1% of the total samples collected. The occurrence of *L. monocytogenes* was highest in vegetables (4.7%) as compared to salad (1.7%) and coleslaw (4.4%) the difference was not statistically significant. This could be attributed to cross contamination as these vegetables are mixed together at sales points or even washed from the same water source.

Although the relationship between vegetable type and occurrence of *L. monocytogenes* was not statistically significant, the relatively highest prevalence of *L. monocytogenes* in cabbage (8.5%) is similar to the findings by Monge and Arias-Echandi (1999) who reported cabbage as the base vegetable for the occurrence of this organism. This could be attributed to the presence of high levels of fermentable sugars such as glucose which can be readily utilized by *L. monocytogenes* (Beauchat et al., 1986; Dogbe, 2010). The occurrence was least in carrot and this agrees with findings by Beuchat and Brackett (1990) who reported the inhibitory effect of raw carrot on *L. monocytogenes* when compared with cooked carrot. Nguyen-the and Lund (1996) also reported the inhibitory effect of carrot juice on *L. monocytogenes*.

Among the 236 salad vegetable samples that were positive for *Listeria* spp., 11 (4.7%) of the salad vegetables obtained from the markets were positive for *L. moncytogenes*. This could be attributed to the fact that the organism has been reported to widely occur in nature and has been isolated from soil (David and Odeyemi, 2007; Ikeh et al., 2010). In an attempt to ensure all year round and increased production of these vegetables, irrigation practices are employed. The source of water for irrigation is usually the streams which could also serve as a source of contamination for the vegetables since these water bodies are subjected to varying degree of abuses that result to contamination. Nwachukwu et al. (2010a) reported the occurrence of *L. monocytogenes* in two lakes in Abia state at 91.67 and 79.17%. Odjadjare et al. (2011) also reported the abundance of human pathogens as well as *Listeria* spp. in waste water used for irrigation

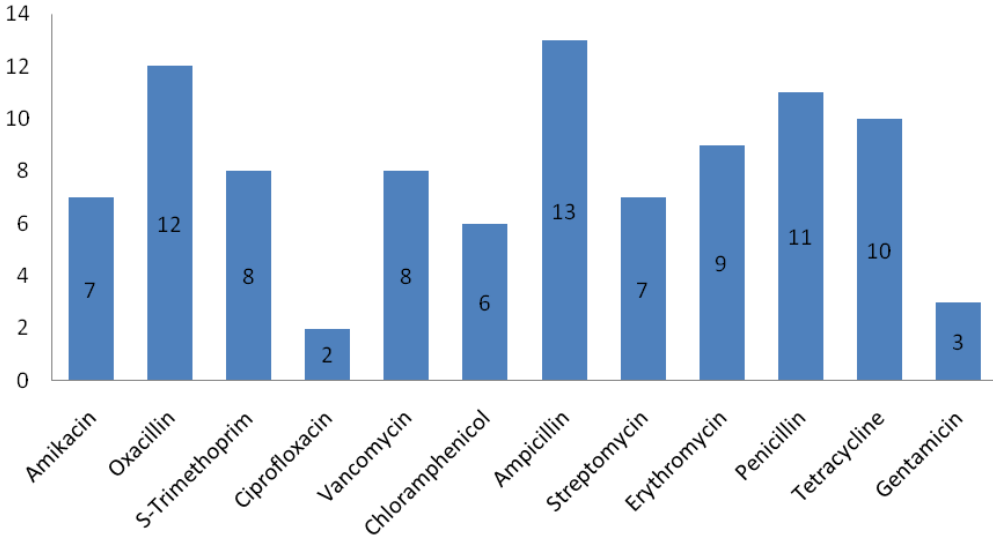

Figure 1. Distribution of the antibiotic susceptibility test of the isolates.

Table 3. Multiple drug resistance of the *L. monocytogenes* isolates.

Antibiotic	Frequency	Percent
Resistance to three antibiotics	4	28.6
Resistance to four antibiotics	1	7.1
Resistance to more than four antibiotics	9	64.3
Total	14	100

in South Africa. Lakes and similar water bodies form the major source water for irrigation and any contaminant in this water bodies could be transferred directly to the vegetables during the irrigation process.

Listeria spp. including *L. monocytogenes* have been isolated from animal dung as well as poultry dropping (Umeh and Okpokwsili, 2009; David and Odeyemi, 2007). These materials serve as a major component of manure that is being applied to the soil to improve yield for agricultural products including salad vegetables. When contaminated, the materials could in turn contaminate all the vegetables on which they are applied thereby acting as a source of the organism.

The high level of antimicrobial resistance of these isolates could be attributed to a sudden increase in the use of antimicrobial agents in treatment of animal diseases, as growth promoters and to the fact that most these antimicrobial agents are not used according to prescription as observed by Yakubu et al. (2012b). The high resistance to ampicillin (92.9%) recorded in this study is similar to that obtained by Issa et al. (2011) and Yakubu et al. (2012b) where 100% resistance to ampicllin were obtained from isolates in milk and processed meat, respectively and Endang et al. (1998) who recorded a similarly high resistance (87.0%) in isolates obtained from salted fish. The finding in this study however is higher than that by Umeh and Okpokwasili (2009) who reported an overall resistance of less than 50% for each of the samples collected. High resistance to oxacillin (85.7%) is also in agreement with the findings by Mauro et al. (2007) who observed that majority of the isolates from meat fish and food processing environment were resistant to oxacillin, lincomycin, flumequine and clindamycin.

The resistance of the isolates against erythromycin (64.3%) is contrary to that obtained by Morobe et al. (2009) in Botswana where all the isolates from various food isolates were reported to be susceptible to erythromycin. Resistance to sulfamethoxazole-trimethoprim (57.1%) and vancomycin (57.1%) were found to be high as compared to 17.1% for Sulfamethoxazole-Trimethoprim and 2.4% for Vancomycin (Wong et al., 2012) as well as 29.82% for Sulfamethoxazole-Trimethoprim (Morobe et al., 2009). Low resistance of *L. monocytogenes* to ciprofloxacin and gentamicin seen in this study has been reported by Yakubu et al. (2012a) and Rahimi et al. (2012) where over 80.0% of isolates from various sources were found to be susceptible to each of these antimicrobial agents.

The multiple drug resistance obtained in this study has also been reported. Yakubu et al. (2012a) reported that 20.0% of the isolates from dairy products were resistant to more than two antimicrobial agents and none of the isolates was resistant to less than one antimicrobial agent which is similar to the result obtained in this study where

100.0% of the isolates were found to be resistant to more than two antimicrobial agents and 64.3% of the isolates were resistant to more than four antimicrobial agents. Lotfollahi et al. (2011) also reported multiple antibiotic resistances in some human isolates of *L. monocytogenes* from blood, urine, vaginal swab, rectal swab and placental bit.

Conclusion

The results obtained from this study have shown that *Listeria* species, and *L. monocytogenes* in particular, are present in salad vegetable and ready to eat vegetable salads sold in Zaria. These findings are of public health significance based on the fact that *Listeria monocytogenes* is cause of listeriosis in animals and human. It was also discovered from this study that cabbage and lettuce pose more public health threat when compared to the other salad vegetables. This requires that cabbage and cucumber need to be washed more thoroughly to reduce their chances of being involved in the transmission of *L. monocytogenes*.

The result also showed that the isolates have multiple drug resistance posing a threat to antimicrobial therapy in the areas of effectiveness of the antimicrobial agents in therapy as well as cost of treatment.

REFERENCES

Abdullahi IO, Abdulkareem S (2010). Bacteriological quality of some ready to eat vegetables as retailed and consumed in Sabon-Gari, Zaria, Nigeria. Bayero J. Pure Appl. Sci. 3:173-175.

Adebayo-Tayo BC, Odu NN, Esen CU, Okonko IO (2012). Microorganisms associated with spoilage of stored vegetables in Uyo Metropolis, Akwa Ibom State, Nigeria. Nat. Sci. 10:23-32.

Adeshina GO, Jibo SD, Agu VE (2012). Antibacterial Susceptibility Pattern of Pathogenic Bacteria Isolates from Vegetable Salad Sold in Restaurants in Zaria, Nigeria. J. Microbiol. Res. 2:5-11.

Beuchat LR, Brackett RE (1990). Inhibitory effects of raw carrots on *Listeria monocytogenes*. Appl. Environ. Microbiol. 56:1734-1742.

Biniam G, Mogessie A (2010). Microbial load, prevalence and antibiograms of *Salmonella* and *Shigella* in lettuce and green peppers. Ethiop. J. Health Sci. 20:41-48.

Chukwu COO (2010). Studies of *Listeria monocytogenes* (bacterium) in Some selected Local Government Areas of Plateau State, Nigeria. University of Jos Repository, Plateau State, Nigeria.

David OM, Odeyemi AT (2007). Antibiotic resistant pattern of environmental isolates of *Listeria monocytogenes* from Ado-Ekiti, Nigeria. Afr. J. Biotechnol. 6:2135-2139.

Dogbe EE (2010). Risk of *Listeria monocytogenes* ingestion in consuming coleslaw purchased from food vendors in the Accra metropolis. A Thesis Submitted to the Department of Nutrition and Food Science of the University of Ghana. pp 184. Available at http://mahider.ilri.org/bitstream/handle/10568/24942/Dogbe.

Endang P, Radu S, Zaiton H, Rusul G (1998). Antimicrobial drug resistance and resistance factor transfer among *Listeria* species. Asian Fish. Sci. 11:261-270.

FAO/WHO (2004). Risk assessment of Listeria monocytogenes in ready-to-eat foods-Technical report by Food and Agricultural Organization/World Health Organization, 2004.

Ikeh MAC, Obi SKC, Ezeasor DN, Ezeonu IM, Moneke AN (2010). Incidence and pathogenicity profile of *Listeria* sp. isolated from food and environmental samples in Nsukka, Nigeria. Afr. J. Biotechnol. 9:4776-4782.

ISO 11290-1 (1996). Microbiology of food and animal feeding stuffs-Horizontal method of detection and enumeration of *Listeria monocytogenes.* Part I: Detection method.

Issa ZM, Mustakim M, Mohamed SAS, Muda NM, Yen LH, Radu S (2011). Antibiogram Profiles of *Listeria monocytogenes* isolated from foods. International Conference on Biotechnology and Food Science IPCBEE. 7:133-137.

Itohan AM, Peters O, Kolo I (2011). Bacterial contaminants of salad vegetables in Abuja Municipal Area Council, Nigeria. Malays. J. Microbiol. 7:111-114.

Janzten MM Navas J, Corujo A, Moreno R, López V and Martínez-Suárez JV (2006). Review. Specific detection of *Listeria monocytogenes* in foods using commercial methods: from chromogenic media to real-time PCR. Spanish J. Agric. Res. 4:235-247.

Lotfollahi L, Nowrouzi J, Irajian G, Masjedian F, Kazemi B, Eslamian L, Falahat A, Ramez M (2011). Prevalence and antimicrobial resistance profiles of *Listeria monocytogenes* in spontaneous abortions in humans. Afr. J. Microbiol. Res. 5:1990-1993.

Manchester A, Clauson A (1995). 1994 spending for food away from home outpaces food at home. Food Rev. 18:12-15.

Mauro C, Domenico P, Vincenzo D, Alberto V, Adriana I (2007). Antimicrobial susceptibility of *Listeria monocytogenes* isolated from food and food-processing environment. *Annali della Facoltà di Medicina Veterinaria di Parma*, XXVII:157-164.

Monge R, Arias-Echandi ML (1999). Presence of Listeria monocytogenes in fresh salad vegetables. Revista Biomédica 10:29-31.

Morobe IC, Obi CL, Nyila MA, Gashe BA, Matsheka MI (2009). Prevalence, antimicrobial resistance profiles of *Listeria monocytognes* from various foods in Gaborone, Botswana. Afr. J. Biotechnol. 8:6383-6387.

NBS (Nigerian Bureau of Statistics, 2012). Consumption Pattern in Nigeria 2009/10. Preliminary Report; March 2012. pp. 71. Available at www.nigerianstat.gov.ng/pages.

Nguyen-the C, Lund BM (1990). The lethal effect of carrot on *Listeria* species. Int. J. Appl. Bacteriol. 70:479-488.

Nwachukwu NC, Orji FA, Iheukwumere I, Ekeleme UG (2010a). Antibiotic Resistant Environmental Isolates of *Listeria monocytogenes* from Anthropogenic Lakes in Lokpa-Ukwu, Abia State of Nigeria. Aust. J. Basic Appl. Sci. 4:1571-1576.

Odjadjare EEO, Igbinosa EO, Okoh AI (2011). Microbial and physicochemical quality of an urban reclaimed wastewater used for irrigation and aquaculture in South Africa. Afr. J. Microbiol. Res. 5:2179-2186.

Pagotto F, Corneau N, Farber JM (2006). *Listeria monocytogenes* infections. In: Riemann, H. P. and Cliver, D. O. (Eds.). Foodborne Infections and Intoxications, 3rd edition, Academic Press. pp. 313-340.

Rahimi E, Momtaz H, Sharifzadeh A, Behzadnia A, Ashtari MS, Zandi ES, Riahi M, Momeni M (2012). Prevalence and antimicrobial resistance of *Listeria* species isolated from traditional dairy products in Chahar Mahal and Bakhtiyari, Iran. Bulg. J. Vet. Med. 15:115−122.

Umeh SI, Okpokwasili GSC (2009). Occurrence and Antimicrobial Profile of *Listeria monocytogenes* in Fresh Feaces of Livestock Animals. Niger. J. Microbiol. 23:1869-1875.

Wong WC, Pui CF, Tunung R, Ubong A, Noor Hidayah MS, Farinazleen MG, Noorlis A, Cheah YK, Son R (2012). Antibiogram Pattern among Cultures of *Listeria monocytogenes* Isolated from Frozen Burger Patties in Malaysia. Pertanika J. Trop. Agric. Sci. 35:793-804.

Yakubu Y, Salihu MD, Faleke OO, Abubakar MB, Junaidu AU, Magaji AA, Gulumbe ML, Aliyu RM (2012b). Prevalence and antibiotic susceptibility of *Listeria monocytogenes* in raw milk from cattle herds within Sokoto Metropolis, Nigeria. Sokoto J. Vet. Sci. 10:13-17.

Yakubu Y, Salihu MD, Faleke OO, Abubakar MB, Magaji AA, Junaidu AU (2012a). Disinfectant effect of Methylated Ethanol against *Listeria* species. Vet. World 5:91-93.