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ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *Listeria* species ISOLATED FROM SOME READY-TO-EAT FOODS SOLD IN KANO, NORTH-WESTERN NIGERIA

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ABSTRACT

The antimicrobial susceptibility profile of L. monocytogenes and other Listeria species isolated from some ready-to-eat (RTE) foods sold in Kano metropolis, north-western Nigeria was carried out using disc-diffusion method. The results obtained showed that L. monocytogenes was moderately susceptible to all the antibiotics tested while L. inovanii and L. seelegeri exhibited high and low susceptibility patterns respectively. However, all the species were resistant to gentamicin, ciprofloxacin, augmentin and ceftriazone. Considering the fact that L. monocytogenes and other Listeria species tested in this study are slowly becoming antibiotic resistant; a situation that poses a threat to both human and animal health, the continuous examination of emerging antimicrobial resistance of these pathogens is important to ensure effective treatment of human listeriosis. The results of this study could therefore help in enriching the data on antibiotic resistance of Listeria strains isolated from foods and in developing effective risk management strategies. In addition, it is recommended that more research be conducted to ascertain the presence of these organisms in other food items in Kano, Nigeria.

Keywords: Listeria species, Ready-to-eat foods, Antimicrobial susceptibility, Kano, Nigeria.

INTRODUCTION

Listeria monocytogenes is a ubiquitous bacterium that is responsible for food-borne illnesses in humans (Griffiths, 1989). It is a bacterial pathogen that contaminates many ready-to-eat (RTE) food products, of which the list includes but not limited to meat products, dairy and dairy products (Hsieh *et al*., 2011; Yan *et al*., 2011), fresh vegetables and fruits (Hsieh *et al*., 2010), fresh sea foods and ready-to-eat foods (Arslan and Ozdemir, 2009; European Food Safety Authority, 2007; Gianfranceschi *et al*., 2007; Bell and Kyriakides, 2005; Schlech, 2000). Consumption of foods contaminated with this pathogen can lead to listeriosis (Conter *et al*., 2009), a disease characterized by symptoms and conditions such as diarrhea, encephalitis and miscarriage in pregnant women. *L. monocytogenes* is a widely-recognized food-borne pathogen that can survive under adverse conditions of temperatures, pH and water activity (USFDA/CFSAN, 2003). In Nigeria, cases of listeriosis and death caused by the disease were not well documented (Umeh and Okpokwasili, 2009). Patients might have died before they could obtain medical help because of inaccessibility in most rural areas, poor state of some health facilities and low level of awareness of *L. monocytogenes* among health professionals and/or absence of the selective laboratory media for isolation due to the high cost of procurement. With particular reference to Kano State, there was lack of information on the occurrence of *L. monocytogenes* in foods until when Aisha and Kawo (2014) as well as Bello (2014) reported the occurrence of *L. monocytogenes* in the State. However, as at the present, the authors have no knowledge of any documentation on listeriosis outbreak in Kano State. In addition, reports have shown that *Listeria* species were susceptible to antibiotics active against Gram-positive bacteria, however, cases of antibiotic resistance in *Listeria* species have been reported in some Asian countries (Rota *et al*., 1996; Walsh *et al*., 2001; Arslan and Ozdemir, 2009; Conter *et al*., 2009). These, among many other reasons, could lead to wrong diagnosis of listeriosis. There appears to be no record of susceptibility pattern of *Listeria* species to some antibiotics commonly obtainable at the market in most clinics/hospitals in Kano. These, among many other reasons, informed the need for this study to be carried out so that proper antibiotics treatment is procured. Therefore, the present study was undertaken to assess the antimicrobial susceptibility pattern of *Listeria* species isolated from some RTE foods sold in Kano, Nigeria to some commonly-used antibiotics. The findings obtained would provide information to evaluate health risks for consumers.

MATERIALS AND METHODS

Isolation and identification of *L. monocytogenes* and other *Listeria* species

L. monocytogenes and other *Listeria* species were isolated from some RTE food samples purchased (December 2012 to March 2013) from areas with high density of RTE and fast-food (FF) joints in Kano metropolis, which included Kabuga, Zoo road, Tarauni, Nassarawa, Bompai road and Municipal areas.

The food samples were transported in insulated cold boxes to the laboratory and analyzed on the same day in accordance with the methods of the United States Food and Drug Administration as well as Center for Food Safety and Applied Nutrition (2003). The study was conducted at the Microbiology Laboratory of the Department of Microbiology and Parasitology, Aminu Kano Teaching Hospital, Kano, Nigeria, after seeking Ethical Permission (Appendix I) from the Hospital's authorities. Here, five (5) grams of the food sample was aseptically taken, blended for 2 minutes in 45 ml sterilized *Listeria* - enrichment broth base + *Listeria* primary-enrichment supplement and incubated at 30°C for 48 hours. A loopful of the broth culture was streaked onto *Listeria* selective agar (LSA) base (Oxford formulation) + *Listeria* selective supplement (Oxoid, Hampshire, UK) and incubated aerobically at 37^{o}C for 24 hours. Colonies that appeared brown-black with a depressed center and a surrounding black halo were taken as positive for *Listeria* species. Typical colonies from each LSA plate that had blackened were streaked onto Triptose soy agar with 0.6% yeast extract (TSAYE) and incubated at 30^{o}C for 24 hours. Isolates were transferred to the sterile slants of triptose soy broth with yeast extract, incubated at 30^{o}C for 24 hours and stored at 4^{o}C for further use (Hitchins, 2003). Heamolysis of the suspected colonies on sheep blood agar plates was also determined. Gram's staining and biochemical screening of the various isolates to specie level were carried out using Microbact 12L (Oxoid, Hampshire, UK) (Plate I) method while results were interpreted using the Microbact™ Identification Package (Windows®) MB1244A (Plate II) (Zhang *et al*., 2007; Hitchins, 2011).

Plate I: Microbact™ 12L (I) (Self – contained biochemical based identification system for the definitive identification of *Listeria* species)

Plate II: Microbact™ Identification Package

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was performed for *L. monocytogenes* and other *Listeria* isolates in accordance with disc-diffusion method using Muller Hinton Agar (NCCLS, 2007). Thirteen (13) commonly-used antibiotics were chosen for the study. They were amoxyclav (10µg), cloxacillin (1µg), cotrimaxazole (25µg), levofloxacin (5µg), erythromycin (15µg), gentamicin (10µg), clindamycin (2µg), ciprofloxacin (10µg), amoxicillin/clavulanic acid (30µg), imipenem (10µg), ceftazidime (30µg), augmentin (10µg) and ceftriaxone (30µg). The method applied for antimicrobial testing was agar plate antibiotic disk diffusion method using Kirby-Bauer technique (MacGowan *et al*., 1990; NCCLS, 2007). Two pure colonies of the isolates were taken from the Tryptose soy agar with yeast extract and suspended in normal saline and then incubated at 37^{0}C for 2 hrs. The suspension was then checked for the development of turbidity against 0.5MacFarland standard. It was inoculated by dipping a sterile cotton swab into it and swabbing on the Muller-Hinton agar. Then, the antimicrobial discs were firmly placed on it and the plates were incubated at 37°C for 24 hrs after which zone of inhibition around each disc (Plates III-IV) was measured using millimeter rule. The results were interpreted as sensitive, intermediate or resistant using a standard zone interpretative chart (NCCLS, 2007). *Listeria monocytogenes* (ATCC 19115) was used as positive (quality) controls (Plate V) for checking the efficiency of both culture media and antimicrobial susceptibility testing methods.

Plate III: Zones of inhibition of some antibiotic disks on Mueller-Hinton agar plate

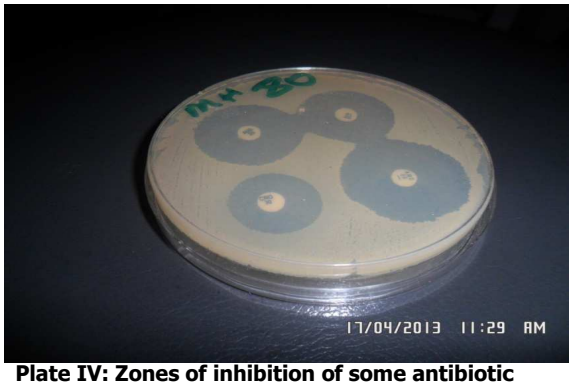

Plate IV: Zones of inhibition of some antibiotic disks on Mueller-Hinton agar plate

Plate V: *Listeria monocytogenes* ATCC 19155 Control strain (Quality Control)

RESULTS AND DISCUSSION

In this study, a total of 336 RTE food samples (from 8 categories of RTE foods) were screened for the presence of *Listeria monocytogenes* and other *Listeria* species of which the prevalence and distribution of these organisms have earlier been reported (Aisha and Kawo, 2014). Table 1 presents the antimicrobial susceptibility profile of the 38 strains of *Listeria* species isolated from the eight (8) categories of RTE food samples examined in this study. The results showed that *L. monocytogenes* was moderately susceptible to all the antibiotics tested while *L. inovanii* and *L. seelegeri* exhibited high and low susceptibility patterns respectively. These results support the earlier findings of Umeh and Okpokwasili (2009) that *L. monocytogenes* was susceptible to a wide range of antibiotics, which included the erythromycin, cotrimoxazole, amoxicillin but resistant to chloramphenicol, augmentin, gentamicin and nalidixic. However, all the species were resistant to gentamicin, ciprofloxacin, augmentin and ceftriazone. Multiple drug resistance by *Listeria* species has been documented in many parts of the world (Wang *et al*., 2012). This resistance could be attributed to the gross misuse of these drugs in chemotherapy particularly in this part of the world. In addition, the observed resistance to some of the tested drugs in this study could be attributed to the earlier report that *Listeria* species can prove difficult to control especially on food contact surfaces such as stainless steel because the bacteria can form persistent biofilms (USFDA/CFSAN, 2003). Table 2 shows the distribution of the *Listeria* species against the tested antibiotics with respect to their individual responses. Resistance to ceftazidime was the most prominent with 36 isolates showing resistance to this antibiotic while the least were cotrimaxazole and levofloxacin each with one (1) isolate only while none was resistant to cloxacillin, amoxicillin/clavulanic acid, augmentin and ceftriaxone. The ease in the procurement of antibiotics and their indiscriminate use prior to coming to the clinic/hospital could be responsible for these observations (Bashir *et al*., 2011). On the other hand, varying degrees of susceptibility were exhibited by the various *Listeria* species against all the antibiotics tested with all the 38 isolates (100%) susceptible to cloxacillin and ceftriaxone while only one (2.64%) was susceptible to ceftazidime. This, of course, is in support of Charpentier *et al*. (1995) who reported that *L. monocytogenes* as well as other *Listeria* species are usually susceptible to a wide range of antibiotics.

In Nigeria, the actual situation of listeriosis is still unknown and little information exists on the occurrence of *L. monocytogenes* in foods consumed in the country. It is also important to note that listeriosis is a disease ignored in Nigerian health system. In addition, there have been no criteria or standards for *L. monocytogenes* in foods in the country. On the other hand, the food habit of Nigerian population is different from other countries. Besides common foods, a significant variety of locally-produced and traditional foods are consumed. Therefore, the first step to persuade regulatory authorities and private manufacturers about the importance of *Listeria* in foods is to provide data on the antibiotic susceptibility pattern of these organisms in various foods.

Table 1: Antimicrobial susceptibility profile of *Listeria* species isolated from ready-to-eat food samples collected from fast-food joints in Kano, Nigeria

Listeria species	AMC (10)	COX (1)	COT (25)	LEV (5)	ERY (15)	GEN (10)	CLD (2)	CIP (10)	ACA (30)	IMI (10)	CEF (30)	AUG (10)	CFX (30)
Listeria monocytogenes													
S	05	06	06	06	04	06	06	06	03	04	00	05	04
I	01	00	00	00	00	00	00	00	01	00	00	01	02
R	00	00	00	00	02	00	00	00	02	02	06	00	00
Listeria inovanii													
S	29	28	30	27	28	31	30	31	25	23	02	30	29
I	00	00	00	02	02	00	00	00	04	06	01	01	02
R	02	03	01	02	01	00	01	00	02	02	28	00	00
Listeria seelegeri													
S	01	01	01	00	01	01	01	01	00	01	00	01	01
I	00	00	00	01	00	00	00	00	01	00	00	00	00
R	00	00	00	00	00	00	00	00	00	00	01	00	00
Control (ATCC 19115)													
S	01	01	01	00	01	01	01	01	01	00	00	01	00
I	00	00	00	01	00	00	00	00	00	01	00	00	01
R	00	00	00	00	00	00	00	00	00	00	01	00	00

Key: AMC = Amoxyclav, COX = Cloxacillin, COT = Cotrimaxazole, LEV = Levofloxacin, ERY = Erythromycin, GEN = Gentamicin, CLD = Clindamycin, CIP = Ciprofloxacin, ACA = Amoxicillin/clavulanic acid, IMI = Imipenem, AUG = Augmentin, CFX= Ceftriazone

Table 2: Overall antimicrobial susceptibility profile of *Listeria* species isolated from ready-to-eat food samples collected from fast-food joints in Kano, Nigeria

Antibiotic	Dose (µg/disc)	Resistant isolates		Intermediate Isolates		Susceptible Isolates	
		Number	Percentage	Number	Percentage	Number	Percentage
Amoxyclav	10	02	5.26	01	2.64	35	92.1
Cloxacillin	1	00	0.00	00	0.00	38	100.0
Cotrimaxazole	25	01	2.64	00	0.00	37	97.4
Levofloxacin	5	01	2.64	00	0.00	37	97.4
Erythromycin	15	03	7.89	00	0.00	35	92.1
Gentamicin	10	03	7.89	02	5.26	33	86.8
Clindamycin	2	04	10.5	06	15.8	28	73.7
Ciprofloxacin	10	04	10.5	07	18.2	27	71.1
Amoxicillin/clavulanic acid	30	00	0.00	02	5.26	36	94.7
Imipenem	10	02	5.26	04	10.5	32	84.2
Ceftazidime	30	36	94.7	01	2.64	01	2.64
Augmentin	10	00	0.00	05	13.2	33	86.8
Ceftriaxone	30	00	0.00	00	0.00	38	100.0

CONCLUSION AND RECOMMENDATIONS

The results of this study demonstrated that a wide range of multi-drug resistant determinants are present in members of the *Listeria* genus with significant potential for transfer to the currently pathogenic species of *L. monocytogenes*. These findings could therefore serve as useful information towards evaluating health risks for consumers and to determine the susceptibility of *Listeria* species isolated from other retail food products in Kano, Nigeria.

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Author's contribution

Kawo A.H. designed the research, supervised it, wrote and edited the accepted manuscript. Bello A.M. conducted the research and read the draft and final manuscripts.

Conflict of interest

There is no conflict of interest.

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APPENDIX I

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AKTH/MAC/SUB/12A/P-3/VI/1108 6th February, 2013

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Ufs:

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ETHICAL APPROVAL

Refer to your application in respect of your research proposal titled "Isolation and Susceptibility Profile of *Listeria monocytogenes* Recovered from some Processed Foods in Kano". The Committee reviewed your proposal and noted same as a Prospective study.

In view of the above, Ethical approval is hereby granted to conduct the research.

However, the Committee requests you to provide an update report of the progress of the study and its completion to the research ethics committee.

Regards

Bara'atu Kabir (Mrs)
Secretary Ethical Committee
For: Chairman