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Antibiotic Resistant Environmental Isolates of *Listeria monocytogenes* from Anthropogenic Lakes in Lokpa-Ukwu, Abia State of Nigeria

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Abstract: Prevalence rates of *Listeria monocytogenes* in two anthropogenic lakes in Abia State, Nigeria were determined. Antibiotic resistance patterns of the isolates were studied by paper disk assay. A total of 48 water samples from the two lakes under study were cultured on listeria selective medium after pre-enrichment. Out of 24 water samples from each of the two lakes, 91.67% and 79.17% were positive for *Listeria monocytogenes* for lake A and B respectively. Drovid was the most effective antibiotics against the pathogen in both lakes while ampiclox and penicillin proved to be least effective with resistance rates of 40.91%; 42.11% and 40.91%; 47.37% for lake A and B respectively. Combined action of antibiotics against the pathogen at invitro level could raise the hope of chemotherapy but multi-drug resistance of the environmental isolates was documented. Governmental intervention is needed for the provision of potable water for people living and surviving partly or wholly through the use of lake water. Experts in environmental health, public health, veterinary medicine, food, microbiology and sociology are needed to intensify aggressive campaign on the dangers of use of lake water in food processing.

Key words: *Listeria monocytogenes*, lake, lake water, antibiotic resistance, environmental isolates, Abia State, Nigeria

INTRODUCTION

Listeria species are considered ubiquitous organisms and are widely distributed in the environment (Seelinger and Jones, 1986). *Listeria monocytogenes* has been recognized for many years as a facultative pathogenic bacterium that causes serious illness in man and animals called listeriosis (Schuchat *et al,* 1982; Flemming *et al.,* 1985; Anon, 1999). Relatively recently, it has been implicated in several food-borne epidemic, and it is also widely distributed in the environment: soil, sewage, raw and decaying vegetables (Seelinger and Jones, 1986).

Listeria monocytogenes remains the principal cause of meningitidis, encephalitis, abortion or septicaemia (Neiman and Lober, 1980). Listeriosis affects most often the pregnant women, foetus, elderly and immuno compromised by affecting the central nervous system (CNS), and blood circulation (Farber and Peterkin, 1991; Ertas and Seker, 2005).

The virulence of *Listeria monocytogenes* has been established in most diversified studies, and reports that a good number, if not all environmental isolates of *Listeria monocytogenes* are virulent in man and animals exist (Gellin and Broome, 1989; Swaminathan *et al.,* 1998).

The pathogen occurs as sporadic cases, and contaminated food, water is the principal vehicle of transmission (Schlech, 1983; Mead *et al.,* 1998). *Listeria monocytogenes* has been identified to be defiant to environmental stresses including low pH, heat of process (pasteurization), high osmotic pressure and refrigeration temperature of 4°C.

In water and soil environment, *Listeria monocytogenes* is able to form biofilms especially if the nutrient conditions are quite favorable (Hood and Zottola, 1997; Stephanovic *et al.,* 2004; Adetunji and Adegoke, 2008).

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Listeria monocytogenes has fair stability over antibiotic susceptibility (Schald, 1983), although an increase in the antibiotic resistance of *Listeria monocytogenes* has previously been reported (Peterkin *et al.,* 1991). In Northern Nigeria and North America, it was reported that most strains of the organism were sensitive to ampicillin, erythromycin and other common antibiotics. Surprisingly, the same research study reported that the organism resisted cephalosporins, nitrofurantoin, tetracycline, chloramphenicol, at invitro levels (Onyemelukwe *et al.,* 1983; Cherubin *et al.,* 1991; Adetunji and Adegoke, 2008).

In Western Nigeria, a multiple-antibiotic resistance in environmental isolates of *listeria monocytogenes* has been reported (David and Odeemi, 2007).

This study investigated the incidence of *L. monocytogenes* in water from two anthropogenic lakes in Abia State, Eastern Nigeria. This study also investigated the susceptibility of the isolates to antimicrobial agents commonly used in chemotherapy in Eastern Nigeria.

MATERIALS AND METHODS

Study Area:

The study area is Lokpa-Ukwu, Umuchieze Community located in Abia North Senatorial Zone of the State in South Eastern Nigeria. The community is 15kilometers from Okigwe along the Port Harcourt-Enugu Expressway, Nigeria. The community lies within latitudes of $5^{o}04$ and $5^{o}03^{1}$ North and longitudes $7^{o}10^{1}$ and $7^{o}35^{1}$ East (Igbozurike, 1983). Umuchieze has a tropical climate and mean daily maximum temperature range from 28 – 35^{o}C (Nwoke and Uwazie, 1991). Wet and dry seasons are distinct in the area. The wet season begins from March through October giving an annual rainfall of between 1700mm – 20,000mm. In habitants of the region use the lake water for a number of domestic activities.

Collection of Samples:

A total of forty-four water samples (24 from Lake A and another 24 from lake B) were collected from the anthropogenic lakes under study. The water samples were collected using sterile universal bottles, and transported within 1hour – 2hours to the central laboratory, Department of Microbiology, Abia State University, Uturu – Nigeria for bacteriological studies. Storage of samples was also carried out at refrigeration temperature and under ice.

Isolation and Identification Procedures:

The culture method was on the basis of international standard (ISO 11290-1-1997) as previously described by Narang, 2004.

25grams of each of the water samples were weighed out into a sterile tube using sterile disposable pipettes and 225ml of Fraser broth (oxiod: cm0895) were added to the lake water samples to form 10^{-1} dilution.

After 24hrs incubation, 1.0ml of the dilution was transferred to a tube containing 10ml of listeria enrichment broth (oxoid, cm0862). The tubes were incubated for 24 – 48hrs at 37^{o}C$\pm$ 2^{o}C. One – two loops of listeria broth culture were streaked onto Listeria Selective Agar (Oxford formulation, oxoid, cm0856), and plates were incubated for 48hrs at 37^{o}C$\pm$ 2^{o}C under microaerophilic conditions. Suspected listeria colonies were identified by biochemical tests including gram stain, motility, oxidase, β-haemolysis, catalase, carbohydrate fermentation tests (Xylose, Mannitol, Rhamnose) and Methyl Red, Voges – Proskaour Reaction as previously described (Seelinger and Jones, 1986; Arora, 2004; Narang, 2004).

Antibiotic Susceptibility:

The antibiotic susceptibility of the isolates was determined by the disk diffusion method on Muellar-Hinton Agar as previously described by Madigan and Martinto, 2006. The antibiotics (Optun Laboratories, Nigeria Ltd) used included: ciprofloxacin (10µg), norflofloxain (10µg), gentamycin (20µg), lincocin (30µg), streptomycin (10µg), rifampicin (30µg), erythromycin (30µg), chloramphenicol (20µg), ampiclox (20µg), floxapen (10µg), and penincillin (10µg).

The inoculum was standardized by adjusting its density to equal the turbidity of Barium sulphate ($BaS0_{4}$), which is the 0.5 McFarland turbidity, and incubated at 37^{o}C± 2^{o}C for 18 hours. The diameter of the zone of clearance (including the diameter of the disk) was measured to the nearest whole millimeter and interpreted on the basis of the interpretive guideline of the National Committee on Clinical Laboratory Standards (NCCLS) now Clinical Laboratory Standard Institute (CLSI) (Table 4).

RESULTS AND DISCUSSION

The identification of isolates was based on cultural and morphological appearances. The identity of isolates was confirmed by biochemical tests. The results of this study revealed that the pathogen was present in 22 out of 24 samples of water from Lake A, giving a prevalence rate of 91.67% while in lake B, the prevalence rate observed was 79.17%. (Table 1)

This high prevalence rate is not surprising as the organism is quite ubiquitous in soil, water, and animal dung samples. *Listeria monocytogenes* is a water and food borne bacteria pathogen that is ubiquitous in nature and shows ability to persist in its environment for prolong time (Yutaka *et al.,* 2004).

Similarly, in a study by Salihu *et al,* (2008) out of a total of 150 smoked fish samples in Sokoto, Nigeria, 25% prevalence rate of *Listeria monocytogenes* was observed. The authors acknowledged that contamination may have resulted from post processing and storage. The low prevalence rate of *Listeria monocytogenes* from smoked fish in Nigeria is not surprising because smoking is done at high temperature, which the organism cannot tolerate.

The isolation of *Listeria monocytogenes* 91.67% and 79.17% times from lakes A and B respectively is of serious public health implication. The inhabitant of Lokpa town, use the lake water for a lot of domestic activities including washing of fresh food items such as fermented cassava (Tapioca), breadfruit, melon, seeds, tomatoes, etc and other vegetables such as *Telfairia occidentalis*, etc. The food preparation culture does not include pickling rather ordinary parboiling culture, which will not knock off this pathogen completely. The infective dose of the organism is very low, and consumption of foodstuffs washed in the lakes becomes a risk factor for transmission of listeriosis.

Salihu *et al,* (2008) also cited that the presence of this pathogen on smoked fish is an indication that the hygiene and safety of such fish is already compromised by the environment.

The presence of *Listeria monocytogenes* in fish has been reported by a number of researchers (Farber and Peterkin, 1991;Nicholas *et al.,* 2000).

In Ado Ekiti, Western Nigeria, *Listeria monocytogenes* has been reported in cow dung, soil and vegetable in prevalence rates of 85%, 91.60% and 73.75% respectively. (David and Odeyemi, 2007). Similarly, David and Odeyemi, (2007) acknowledged that the cow dung which is regularly used by farmers in the region to fertilize farmlands contaminates the vegetables grown in such lands and it is transmitted to man and animals upon consumption.

In addition, in Nigeria, researchers have isolated *Listeria monocytogenes* from meats and poultry droppings applied on farmlands (Chukwu *et al.,* 2004; Chukwu *et al.,* 2006) and cited the grave consequences of the human infection through animal products and vegetables.

Environmental isolates of the organism showed resistance to different antibiotics. Isolates from lake A were resistant to ciprofloxacin (36.36%), norfloxacin (18.18%), gentamycin (22.72%), lincocin (22.72%), streptomycin (22.72%) and rifampicin (22.22%), erythromycin (27.27%), chloramphenical (27.27%), ampiclox (40.91%), floxapen (18.18%), penincillin (40.91%) and drovid (13.63%). Isolates from lake b were resistant to ciprofloxacin (21.05%), norfloxacin (26.32%), gentamycin (36.84%), lincocin (31.57%), streptomycin (31.57%), rifampicin (26.32%), erythromycin (27.27%), chloramphenicol (42.11%), ampiclox (42.11%), floxapen (21.05%), penincillin (47.37%) and drovid (21.05%). (Table 2).

The prevalence rate of resistance of ciprofloxacin in lake A (36.36%) was significantly greater than ciprofloxacin resistance in lake B.

The resistance shown by *L. monocytogenes* strains from anthropogenic lakes in Nigeria to community used antibiotics may be due to abuse of drug use in human chemotherapy, and escape of selected strains into the water bodies as lakes, streams, oceans etc (Dina and Arowolo, 1991)

Emergence of antibiotic resistant environmental isolates of *Listeria monocytogenes* in Nigeria is of considerable medical and public health significance because of the ability of the organisms to interact with man to initiate listeriosis, which will defy almost all the antibiotic chemotherapy. In strict agreement to this study, (Poyart-Salmeron *et al.,* 1990) reported resistance of *Listeria monocytogenes* strains from a patient with Meningoencephalitis to chloramphenicol, erythromycin and streptomycin. This suggests genetic similarities with the strains used in this study.

L. monocytogenes used in this study were highly resistant to penicillin and ampiclox in both lakes. The widespread use of penicillin and ampiclox in human and veterinary therapy, alongside the length of time over which they have been available in Nigeria and other countries of the world could account for this high resistance.

Environmental factors like previous exposure, type of antibiotic used in the respective locality, incidence of plasmids in the isolates may have resulted to the antibiotic resistance.

Plasmid Acquisation:

This has also been recognized in most isolates of *L. monocytogenes* (Hadorn *et al.,* 1993; Yutaka *et al.,* 2004). The resistance to antibiotics can also be as a result of selective antibiotic pressure (Hanchung *et al.,* 2004).

Multiple antibiotic resistances are summarized in the table 3. Ten different antibiotics were combined, and multiple drug resistance was defined by the ability of an isolate to resist at least two antibiotics of different classes.

In both lakes, combination of quinolones and aminoglycoside decreased the resistance chances, as the percentage resistance rates were 13.64% and 15.78% for lakes A and B respectively. Worthy of note is the fact that single use of quinolones and aminoglycosides gave resistance rates between 13.63% - 40.91% and 21.05% - 47.37% respectively. Visual comparison of the last two statistics might suggest that combined therapy will improve the clinical recovery rates of listeriosis patients. This little increase in susceptibility of pathogen when antibiotics were combined could be refered to as additive effect of combined therapy at invitro level.

In Ado Ekiti, Nigeria, (David and Odeyemi, 2007) isolated multiple antibiotic resistant *L. monocytogenes* from cow dungs, and vegetables. The study in Ado-Ekiti showed that augumentin and amoxicillin combination welded the highest rate of resistance (33.3%). The authors suggested that multi-drug resistance is capable of complicating the management of clinical listeriosis, and that plasmid regulated resistance will usually be transferred to other organisms co-operating with *Listeria monocytogenes* to establish diseases.

The epidemiology of listeriosis in human and veterinary medicine is better understood and controlled through source of infection. The interaction of *Listeria monocytogenes* in the lakes in Lokpa town with inhabitants of the environment could establish listeriosis.

Lack of adequate and well equipped clinical and laboratory diagnostic procedures in Nigeria and lack of report indicating epidemiological link of *L. monocytogenes* and man through ingested food that were partly prepared using the lake water may be contributory factor to the poor attention the disease has received in time past. The presence of this pathogen on smoked fish is an indication that the hygiene and safety of food such as vegetables eaten raw or just parboiled is compromised.

Government on its own should endevour to provide potable water, to inhabitants of Lokpa town to enable them use potable water in food processing. The attention of Environmental Health Scientists, experts in Public Health, Medical and Environmental Sociology, Epidemiology is greatly required in the campaign against the transmission of listeriosis through food processing and environmental contact.

Table 1: Occurrence of *Listeria monocytogenes* in Anthropogenic Lakes in Lokpa-Ukwu Umuchieze, Abia State

Lake of Number Examined	Number Positive	% of Prevalence
Lake A (n = 24)	22	91.67
Lake B (n = 24)	19	79.17

Number of samples positive for listeria monocytogenes.

Table 2: Antibiotic susceptibility of environmental isolates of *Listeria monocytogenes* from anthropogenic lakes in Lokpa-Ukwu, Umuchieze, Abia State

Antibiotics	Lake A	Lake B	P-Values
	N = 22	N = 19	
Ciprofloxacin (10µg)	8 (36.36)	4 (21.05)	0.05
Norfloxacin (10µg)	4 (18.8)	5 (26.32)	NS
Gentamycin (10µg)	5 (22.72)	7 (36.84)	NS
Lincocin (20µg)	6 (27.27)	6 (31.57)	NS
Streptomycin (10µg)	5 (22.72)	6 (31.57)	NS
Rifampicin (20µg)	5 (22.72)	5 (26.32)	NS
Erythromycin (30µg)	6 (27.0)	6 (31.57)	NS
Chloramphenicol (30µg)	6 (27.27)	8 (42.11)	0.01
Ampiclox (20µg)	9 (40.91)	8 (42.11)	NS
Floxapen (20µg)	4 (18.18)	4 (21.05)	NS
Penicillin (10µg)	9 (40.91)	9 (47.37)	NS
Drovid (10µg)	3 (13.63)	4 (21.05)	NS

Numbers in brackets are in percentage, NS-not significant. P-values ≤ 0.05 are significant

Table 3: Multi-Drug Resistant Isolates of *Listeria monocytogenes* from anthropogenic Lakes at Lokpa-Ukwu, Umuchieze, Abia State.

Antibiotics	Lake A	Lake B
	N = 22	N = 19
Cip + Gen	3 (13.64)	4 (15.78)
Cip + Str	4 (18.18)	3 (15.78)
Rif + Chl	6 (27.27)	4 (21.05)
Cip + Ery + Lin	5 (22.72)	7 (36.84)
Pen + Ery	6 (27.27)	4 (21.05)
Pen + Ery + Flo	5 (22.72)	2 (10.52)
Flo + Pen + Drv	6 (27.27)	5 (26.31)

Numbers in brackets are in percentage
Cip = Ciprofloxacin (10µg/disc)
Gen = Gentamycin (10µg/disc)
Str = Streptomycin (10µg/disc)
Rif = Rifampicin (20µg/disc)
Ery = Erythromycin (30µg/disc)
Pen = Penincillin (10µg/disc)
Flo = Floxapen (20µg/disc)
Lin = Lincocin (20µg/disc)
Chl = Chloramphenicol (30µg/disc)
Drv = Drovid (10µg/disc)

Table 4: Interpretative standards for disc diffusion antimicrobial susceptibility testing/Result

Antibiotic	Amount on Disk	Inhibition Zone Diameter (mm)		
		Resistant	Intermediate	Susceptibility
Ciprofloxacin	10µg	14 or less	15 – 16	17 or more
Norfloxacin	10µg	14 or less	15 – 16	17 or more
Gentamycin	20µg	12 or less	13 – 14	15 or more
Lincocin	30µg	14 or less	15 – 18	19 or more
Streptomycin	10µg	6 or less	7 – 9	10 or more
Rifampicin	30µg	13 or less	14 – 22	23 or more
Erythromycin	30µg	13 or less	14 – 22	23 or more
Chloramphenicol	20µg	12 or less	13 – 17	18 or more
Ampiclox	20µg	13 or less	14 – 16	17 or more
Floxapen	10µg	13 or less	14 – 20	21 or more
Penicillin	10µg	14 or less	-	20 or more

National Clinical Committee on Laboratory Standards (NCCLS) defined the standards used. MM: millimeter. Not indicated

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