Listeria Species And Antimicrobial Resistance Profiles Of HIV/AIDS Patients In Lagos, Nigeria.

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Abstract: *Listeria monocytogenes* and other *Listeria* species, as bacterial opportunistic infectious agents in HIV/AIDS patients, are rarely studied prospectively, particularly in developing nations of the world like Nigeria. Furthermore antibiotic susceptibility data of *Listeria* are scarce, and from the initial observation of *Listeria* as being generally antibiotic susceptible, its resistance to antibiotic agents has gradually evolved over the years. The incidence and antimicrobial resistance profile of *Listeria monocytogenes* and other *Listeria* species in HIV/AIDS patients in Lagos, Nigeria were therefore investigated. Fecal samples from 326 HIV/AIDS patients and 200 HIV sero-negative gastroenteritis patients (control) of Lagos State University Teaching Hospital, Lagos, Nigeria, were screened for the presence of 6 different species of *Listeria* and the antibiotic resistance profile of the isolates were determined by the disc diffusion method of antibiotic susceptibility test. There was a statistically significant difference in the incidence of *Listeria monocytogenes* (8.9%) and *Listeria* species (23.3%) in HIV/AIDS patients compared with the incidence of (1%) and (5.5%) respectively in HIV sero-negative patients (control). This finding agrees with previous ones which have identified immune-suppressive status in HIV/AIDS patients as a risk factor of the disease, listeriosis. The antimicrobial resistance profile of the isolates revealed marked resistance to antibiotics tested in varying degrees. The implication of this in deciding the drug of choice for the treatment of the disease listeriosis, and also the status of *Listeria monocytogenes* as a vector candidate in HIV/AIDS vaccine research, in view of the emerging resistance of the organism to antibiotics, were discussed.
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Introduction:

Listeria species are food-borne, Gram-positive, intracellular and zoonotic bacteria. They are diversely present in the environment from where they infect animals and human (Rebiaglati *et al*, 2009, Okwumabua *et al*, 2005).

They consist of *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria innocua*, *Listeria welchimeri*, *Listeria seeligeri*, *Listeria innocua* and *Listeria grayi* (Liu, 2006). *Listeria rocourtie and Listeria marthii* are recently discovered additional species (den Baker *et al*, 2010).

Listeria is the etiological agent of the disease listeriosis, which is characterized by gastroenteritis, septicemia, meningitis, meningo-encephalitis and abortion in pregnant women. Only *Listeria monocytogenes* and *Listeria ivanovii* are generally known to be pathogenic. *Listeria monocytogenes* is known to be pathogenic to both humans and animals, while *Listeria ivanovii* is generally regarded as pathogenic, only to animals. However, there are reported sporadic cases of human infections with *Listeria ivanovii, Listeria seeligeri*, *Listeria innocua* and *Listeria welchimeri* (Rocourt *et al*, 1986, Andre & Genicot,1987, Perrin *et al*, 2003).

Most of the morbidity and mortality that occur in HIV/AIDS patients results from opportunistic infectious agents which take advantage of the lowered cellular and humoral defences of the patient (Singh *et al*, 2003). *Listeria monocytogenes*, and other *Listeria* species are some of the bacterial opportunistic infectious agents in HIV/AIDS patients (Decker *et al,* 1991, Mascola *et al* 1995, Patil *et al,* 2007). Other groups of people susceptible to *Listeria* include new-born children, the elderly, pregnant women and malignant patients on immune-suppressive drugs (Franciosa *et al,* 2001, Ramaswany *et al*, 2007).

Reports, both in sub Saharan Africa and industrialized countries have shown that listeriosis is relatively rare in HIV/AIDS, among immune-deficient population (Emele, 2000, Becondi *et al*, 2006, Patil *et al*, 2007). However, some studies have also shown that the incidence of listeriosis in people with the simple HIV infection was 9 times greater than in the general populations and the incidence in those with AIDS was almost 100 times greater (Mascola *et al,* 1995).

Antimicrobial resistance has generally undergone near exponential increase in the past decades (Safdar & Armstrong, 2003). Prophylactic use of common broad spectrum antibiotics as well as empirical pre-emptive therapy in high risk settings, or indiscriminate usage,

particularly in developing nations, has further accentuated this trend. (Safdar & Armstrong, 2003, Boudarianzadeh, 2007).

However, antimicrobial resistance data of *Listeria monocytogenes* and other *Listeria* species, particularly in developing nations like Nigeria are scarce (Richet *et al*, 2001, Granier *et al*, 2011). From the initial observation of *Listeria* species as being generally antibiotic susceptible, resistance of these organisms to antibiotics have gradually evolved over the years (Walsh *et al* 2000, Morvan *et al*, 2010, Adetunji and Olaoye, 2012).

Listeria monocytogenes, and other *Listeria* species in clinical samples have hitherto, been rarely studied in Nigeria and other developing areas of the world. There are few reports of studies on *Listeria monocytogenes* from Africa (Mbata *et al*, 2008, Ennaji *et al*, 2008, Salihu *et al*, 2008, Adetunji and Olaoye, 2012). These few reports have been on *Listeria monocytogenes* from animal and food sources. This prospective study, to the best of our knowledge is the first known reported prospective study on *Listeria monocytogenes* and other *Listeria* species in HIV/AIDS patients in Nigeria.

Materials and Methods

Ethical Compliance: Ethical permission was sought and obtained from the Lagos State University Teaching Hospital Ethical Committee for the collection of fecal samples from HIV/AIDS patients of HIV/AIDS clinic as well as the HIV sero-negative gastroenteritis patients of the hospital, as control. Consent of the patients were obtained before collection of sample. The samples were brought into the laboratory for immediate analysis.

Collection of samples

Three hundred and twenty-six (326) HIV/AIDS patients from the HIV/AIDS clinic of Lagos State University Teaching Hospital, Ikeja, Nigeria, were screened for the presence of *Listeria* species bacteria in their fecal samples between February 2012 and January 2013. Furthermore, 200 HIV sero-negative gastroenteritis patients from the same Teaching Hospital were similarly screened within the same period as control, for the presence of *Listeria* bacteria in their feces. Samples were brought into the laboratory and cultured immediately.

Isolation and Identification of *Listeria*

One gram of each fecal sample was of emulsified in 10mls of Listeria Enrichment Broth (Oxoid, England) and aerobically incubated in 37^0c incubator for 24hrs. After overnight incubation, 0.1ml of the broth culture was streaked on Listeria Selective Agar (Oxoid, England) plate and incubated in 37^0 c incubator for 24 - 48hrs.

The plates were examined for typical black colonies of *Listeria* species on the Listeria Selective Agar. The suspected colonies were confirmed and further identified by standard identification and biochemical tests including Gram stain microscopy, motility at 25^0c and 37^0 c, catalase test, acid production from glucose, xylose, rhamnose,mannitol, alpha methyl-D- mannoside, esculin hydrolysis, nitrate reduction, beta-haemolytic activity and CAMP test (Rahimi *et al*, 2012).

Antibiotics Susceptibility Testing

Antibiotics Susceptibility Test was performed for the *Listeria* isolates using the disc diffusion technique as described by Bauer *et al* (1996). Antibiotic multidisc and single discs (Abtek, U.K), containing amoxicillin (5ug), chloramphenicol(10ug), Gentamycin(10ug) streptomycin (25ug) cotrimoxazole(25ug), ceftriazone (10ug) ciprofloxacin(10ug), ofloxacin(10ug), pefloxacin (10ug), augmentin(30ug), tetracycline (25ug) and erythromycin(5ug) were employed. The zones of inhibition observed for the *Listeria* isolates were compared with that of reference organism, *Escherichia coli* NCTC 10418 to determine which of the test isolates was resistant or susceptible to the antibiotics tested.

Statistical Analysis

Differences in the *Listeria* species profiles of HIV/AIDS patients and the HIV sero-negative control patients as well as the differences in the antibiotics resistance profiles, were subjected to statistical analysis, using the 'Chi squared' as well as the 'Analysis of Variance' (ANOVA) tests to determine significance or non- significance of differences at 5% level.

Result

A total of 76 out of 326 (23.3%) of HIV/AIDS patients were positive for *Listeria* species out of which 29 (8.9%) were positive for *Listeria monocytogenes*. On the other hand, 11 out of 200 (5.5%) of HIV sero-negative control patients were positive for *Listeria* species, from which 2 (1%), were specifically positive for *Listeria monocytogenes*. The differences in the incidence of *Listeria* species and *Listeria monocytogenes* in the 2 groups of subjects were statistically significant at 5% level ($p < 0.05$). (Table1). In the same vein, differences in the 6 different types of *Listeria* species profiles of the two groups were also statistically significant ($p < 0.05$). (Table2). While the incidence of *Listeria monocytogenes* in HIV/AIDS patients (8.9%) was the highest of the 6 *Listeria* species, the incidence of *Listeria* grayi (0.9%) was the lowest.On the other hand, the incidence of *Listeria innocua* and *Listeria welchimeri* (both 2%) were the highest in the HIV sero-negative control patients while the incidence of *Listeria seeligeri* and *Listeria grayi* were the lowest (both at 0%) (Table2).

Table 1. Detection of Listeria species in HIV/AIDS patients and HIV sero – negative control patients

Sample	No of samples	Number (%) positive for *Listeria* sp.	Number (%) positive for *Listeria monocytogenes*
HIV/AIDS Patients	326	76(23.3%)	29(8.9%)
HIV negative control	200	11(5.5%)	2(1. 0%)

Table 2. *Listeria* species profiles in 326 HIV/AIDS patients and 200 HIV sero-negative control patients.

Listeria species	No (%) of positive samples in HIV/ AIDS Patients	No (%) samples in HIV negative controls
Listeria monocytogenes	29(8.9%)	2 (1.0%)
Listeria innocua	24 (7.4%)	4(2.0%)
Listeria welchimeri	12 (3.7%)	4(2.0%)
Listeria seeligeri	4(1.2%)	0 (0%)
Listeria grayi	3(0.9%)	0(0%)
Listeria *ivanovii*	4(1.2%)	1(0.5)
Total	76(23.3%)	11 (5.5%)

The Antimicrobial Resistance Profile of *Listeria* from HIV/AIDS patients (Table 3) and HIV sero-negative control patients (Table 4) revealed marked resistance of isolates to the antibiotics tested in varying degrees.There is however no statistically significant difference between the resistance level of the *Listeria* species isolates from both groups ($p>0.05$). Nevertheless, the resistance of *Listeria innocua* is the highest ($p<0.5$) when the resistance rate of *Listeria monocytogenes, Listeria welchimeri* and *Listeria innocua* were compared.

Table 3. Antimicrobial resistance profile of *Listeria* isolated from HIV/AIDS Patients

Antimicrobial Agents	*Listeria* species N=76	*L. monocytogenes* N=29	*Listeria innocua* N=24	*L.welchimeri* N=12	*L. ivanovii* N=4	*L. seeligeri* N=4	*L. grayi* N= 3
Amoxicillin	76(100%)	29(100%)	24(100%)	12(100%)	4(100%)	4(100%)	3(100%)
Augmentin	73(96.1%	29(100%)	24(100%)	12(100%)	2(50%)	1(25%)	3(100%)
Tetracycline	42(55.3%	13(44.8%)	17(70.8%)	7(58.3%)	1(25%)	2(50%)	2(66.7%)
Chloramphenicol	48(63.2%	18(62.1%)	18(75.0%)	5(41.7%)	3(75%)	3(75%)	1(33.3%)
Gentamycin	28(36.8%	3(10.3%)	19(79.1%)	2(16.7%)	2(50%)	1(25%)	1(33.3%)
Streptomycin	21(27.6%)	90(31.0%)	8(33.3%)	2(16.7%)	1(25%)	1(25%)	0(0.0%)
Cotrimoxazole	56(73.7%)	23(79.3%)	16(66.7%)	8(66.7%)	2(50%)	3(75%)	4(100%)
Ceftriaxone	70(85.2%)	29(100%)	24(100%)	60(50%)	4(100%)	4(100%)	3(100%)
Ciprofloxacin	22(29.0%)	9(31.0%)	8(33.3%)	2(16.7%)	1(25%)	2(50%)	0(0.0%)
Ofloxacin	14(18.4%)	5(17.2%)	7(29.2%)	2(16.7%)	0(0.0%)	0(0.0%)	0(0.0%)
Pefloxacin	28(36.8%)	13(44.8%)	11 (45.8%)	2(16.7%)	1(25%)	1(25%)	0(0.0%)
Erythromycin	37(48.7%)	14(48.3%)	16 (66.7%)	4(33.3%)	1(25%)	2(50%)	0(0.0%)
Resistance to 1 antimicrobial	-	-	-	-	-	-	-
Resistance to 2 antimicrobials	3(3.9%)	-	-	1(8.3%)	2(50%)	-	-
Resistance to 3 antimicrobials	-	-	-	-	-	-	
Resistance to 4 antimicrobials	25(32.9%)	7(24.1%)	(37.5%)	6(50%)	-	-	3(100%)
Resistance to > 4 antimicrobials	48(63.2%)	22(75.9%)	15(62.5%)	5(41.7%)	2(50%)	4(100%)	-

Table 4: Antimicrobial Resistance Profile of Listeria Isolates from HIV Sero-negative Control Patients.

Antimicrobial	*Listeria species*	*L.monocytogenes*	*L.innocua*	*L.welchimeri*	*L.ivanovii*	*L.seeligeri*	*L.grayi*
Agent	n=11	n=2	n=4	n=4	n=1	n=0	n=0
Amoxicillin	11(100%)	2(100%)	4(100%)	4(100%)	1(100%)		
Augmentin	11(100%)	2(100%)	4(100%)	4(100%)	1(100%)		
Tetracycline	6(54.5%)	1(50%)	3(75%)	2(50%)	0(0%)		
Chloramphenicol	8(72.7%)	1(50%)	3(75%)	3(75%)	1(100%)		
Gentamycin	5(45.5%)	0(0%)	2(50%)	2(50%)	1(100%)		
Streptomycin	6(54.%)	1(50%)	3(75%)	2(50%)	0(0%)		
Cotrimoxazole	8(72.7%)	1(50%)	3(75%)	3(75%)	1(100%)		
Ceftriaxone	8(72.7%)	2(100%)	4(100%)	2(50%)	0(0%)		
Ciprofloxacin	5(45.5%)	1(50%)	1(25%)	2(50%)	1(100%)		
Ofloxacin	3(27.3%)	0(0%)	1(25%)	2(50%)	0(0%)		
Pefloxacin	7(63.6%)	1(50%)	3(75%)	2(50%)	1(100%)		
Erythromycin	9(81.2%)	2(100%)	3(75%)	3(75%)	1(100%)		
Resistance to							
1 antimicrobial	-	-	-	-	-		
Resistance to							
2 antimicrobials	1(9.1%)	-	-	1(25%)	-		
Resistance to							
3 antimicrobials	2(18.2%)	1(50%)	-	1(50%)	-		
Resistance to							
4 antimicrobials	1(91.1%)	1(50%)	-	-	-		
Resistance to							
5 antimicrobials	7(63.6%)	-	4(100%)	2(50%)	1(100%)		

Resistance to amoxicillin was highest for *Listeria* species and *Listeria monocytogenes* in both groups of subjects at 100%. Similar results were obtain for augmentin in both groups except that resistance is slightly lower (96.1%) in *Listeria* species isolates of the HIV/AIDS patients.The lowest resistance rates of 17.2% and 10.3% were observed in ofloxacin and gentamycin respectively, for *Listeria monocytogenes* isolates from HIV/AIDS patients. Similar results of 0% were observed with both ofloxacin & gentamycin for HIV sero-negative control patients.

Multi-resistance (resistance to greater than 4 antimicrobials) of isolates were observed in both HIV/AIDS patients and HIV sero-negative control patients at (63.2%) and (63.6%) respectively. (Tables 3 and 4).

Discussion

This study investigated the incidence and antimicrobial susceptibilities of *Listeria* species in HIV positive patients in Lagos, Nigeria. Most of the previous reported studies on *Listeria* in HIV/AIDS patient are case studies (Decker *et al*, 1991, Cummins *et al*, 1994, Norberg *et al*, 2004, Tsal *et al* 2006, Low *et al*, 2007, Goborodko *et al*, 2011, Gunst and Jensen-Fangel, 2014), while others are reviews and retrospective studies (Ortel, 1989, Kales and Hotzman,1990, Jurado *et al*, 1993, Mascola et al, 1995, Patl *et al*, 2007,). Prospective studies on *Listeria* in HIV/AIDS patients are rare and this study, to the best of our knowledge is the first known reported prospective study on *Listeria* in HIV/AIDS patients in Nigeria. Moreover, studies have been conducted mostly on *Listeria monocytogenes* than any of the other *Listeria* species, probably because only *Listeria monocytogenes* out of all the *Listeria* species, is considered to be pathogenic to human and animals, while *Listeria ivanovii* is considered to be pathogenic, only to animals(Liu, 2006, Law & Donachie,1997). However, there have been reported cases of other *Listeria* species like *Listeria ivanovii*, *Listeria welchimeri, Listeria innocua* and *Listeria seeligeri* causing disease in both immune-competent and immune-suppressive individuals (Rocourt *et al*, 1986,

Perrin *et al*,2003). This informed the extension of the scope of this study to other *Listeria* species in HIV/AIDS patients, who because of their immunosuppressive status are more susceptible to opportunistic pathogens among which *Listeria* species have been incriminated (Mascola *et al,* 1995, Patil *et al*, 2007).

The results of this study confirms that HIV infection is a risk factor of listeriosis in Nigeria as in other nations of the world (Mascola *et al,* 1995) given the statistically significant ($p<0.05$) higher incidence of *Listeria* sp. (23.3%) and *Listeria monocytogenes* (8.9%) in HIV/AIDS patients when compared with the incidence in HIV sero-negative control patients (5.5% and 1.0% respectively). The incidence of *Listeria monocytogenes* in HIV positive individuals is known to be about 9 times more than in HIV sero-negative individuals (Mascola *et al*,1995) and our results compare favorably and agree with this finding. Furthermore, our results reveals the incidence of each the 6 *Listeria* species and discovered that in Lagos, Nigeria, while the incidence of *Listeria monocytogenes* in the HIV patients is the highest (8.9%), it is closely followed by that of *Listeria innocua* (7.4%), while *Listeria grayi* has the lowest incidence at 0.9%.

The antimicrobial resistance profile of *Listeria* in this study reveals massive multiple antibiotics resistance of many of the isolates to the antibiotics tested. High level of antimicrobial resistance of bacterial pathogen associated with diarrhea is known to be common in many areas of the developing world (Jankelevich, 2006). This study also confirms the steady state of emergence of *Listeria* species from food and clinical cases of listeriosis, resistant to one or multiple antibiotics, since 1990 when the first antibiotic resistant strain of *Listeria monocytogenes* was reported (Poyart Salmeron *et al*, 1990, Zhang *et al*, 2007).

The high resistance profile of *Listeria* in this study is in conformity with the findings of Adetunji and Olaoye, (2012), where similar high resistance profile was discovered of *Listeria monocytogenes* isolates of goat milk in south-west Nigeria. Another study on *Listeria monocytogenes* from raw meat and meat product in Zaria, north-central Nigeria, revealed that all twelve isolates investigated were resistant to 9 antimicrobial agents out of 14 agents tested, but the isolates were sensitive to gentamycin(Yakubu et al, 2012). This zero resistance level of gentamycin to *Listeria monocytogenes* is similar to that obtained in this study where resistance of *Listeria monocytogenes* to gentamycin was the lowest (10.3%) out of the 12 antibiotics tested. This confirms that gentamycin is also the drug of choice in the treatment of listeriosis in Nigeria. However, its suitability in combination with amoxicillin for synergistic purpose needs to be further investigated and evaluated, given the 100% resistance of isolates to amoxicillin as obtained in this study. The statistically significant highest resistance rate of *Listeria innocua,* when compared with *Listeria* monocytogenes and *Listeria welchimeri* agrees with the findings of Morvan *et al*, (2010) that *Listeria innocua* is more resistant than *Listeria* monocytogenes (Morvan *et al*, 2010).

This study is significant, not only from the point of establishing *Listeria monocytogenes* and *Listeria* species as opportunistic disease agents in HIV/AIDS patients, but also from the point of view of the status of *Listeria monocytogenes* as a vector agent in HIV vaccine research (Lieberman and Frankel, 2002, Starks *et* al, 2004, Shollenberger *et al*, 2013). One of the attributes of *Listeria monocytogenes* that dictates its candidature as a vector agent in HIV vaccine research was its susceptibility to a wide range of commonly used antibiotics. It was thought that this antibiotic susceptibility characteristic would make for easy treatment in the event that the subjects of such a vaccine trial develop infections with the vector (i.e. *Listeria monocytogenes*). The findings of this present study, as well as other previous findings of marked resistance of *Listeria monocytogenes* to antimicrobial agents therefore poses a concern as to its suitability as a vector agent in HIV/AIDS vaccine research.

The results of this study also serve as a template for more studies on the clinical strains of *Listeria monocytogenes* and other *Listeria* species for further epidemiological, public health and antimicrobial resistance surveillance in Nigeria. Furthermore, the genetic basis of the multi-resistance of Nigerian clinical strains of *Listeria* to antibiotics needs to be investigated as parts of the efforts aimed at combating or controlling the problem of antibiotics resistance of bacterial pathogens.

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