# MULTIDRUG-RESISTANT *Citrobacter* SPECIES IN PREGNANT HIV OUTPATIENTS ON ANTIBIOTIC THERAPY AT NIGERIA AIR FORCE HOSPITAL, KONJO, NIGERIA

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**Abstract:** An individual may harbour other asymptomatic infections different from the presumptively diagnosed pathogen. Infection, pregnancy or inadequate antibiotic intakes tend to increase human susceptibility to opportunistic bacteria. For this reason, this present study was designed to identify via cultivation and isolation, *Citrobacter* and *Listeria* species (spp.) in pregnant HIV-positive female outpatients of Nigeria Air Force Hospital in Konjo area of Makurdi and evaluate their antibiogram. Eighty-two (82) urine samples obtained from these outpatients, who were on antibiotic therapy, were cultured on nutrient, Cysteine lactose electrolyte deficient and eosin methylene blue agar to isolate *Citrobacter* and *Listeria* spp. Our results show that in both categories of females, the occurrence of *Citrobacter* spp. was more than that of *Listeria* species. Distinctively, pregnant women harboured more of both pathogens than non-pregnant females with the former having more (66.7%) of *Citrobacter* than *Listeria* species (33.3%). In the two age groups, positive cultures of *Citrobacter* spp. were higher than those of *Listeria* spp. However, both infections were higher (38.7%) in young adults that were between 18 and 30 years than in older adults (27.5%) that were above 50 years of age. In the antibiogram studies, *Citrobacter* spp. were resistant to all the tested antibiotics (ciprofloxacin, trimethoprim, erythromycin, pefloxacin, cefalexin, amoxicillin, ampicillin, augmentin, ofloxacin, spiramycin) while *Listeria* spp. were only susceptible to pefloxacin (13mm) and cefalexin (15mm) and antibiotic-not-susceptible to all othes. From the fore-going, since *Citrobacter* species are not susceptible to these commonly used antibiotics, it is advisable to carry out their proper culture and sensitivity to antibiotics before the commencement of any antibiotic medication especially in pregnant women or if on empiric therapeutic use, allow for a change in the choice of antibiotics.

**Keywords:** Cultivation, isolation, pregnancy, HIV, *Citrobacter*, *Listeria*, antibiogram

## Introduction

Pregnancy, infections such as HIV, and the administration of sub-curative doses of antibiotics either through self-medication or non-adherence to dosage regimens, encourage the growth of opportunistic pathogens and development of complicated UTIs especially in developing countries where the provision of health services is either limited or unavailable (Niemogha *et al*., 2006; Barnie *et al*., 2019; Fonsah *et al*., 2017).

*Citrobacter*, a distinct genus of aerobic, Gram-negative bacilli of the *Enterobacteriaceae* family that is widely distributed in water, soil, food and intestinal tract of man and animals are emerging pathogens causing septicaemia, gastroenteritis, neonatal meningitis, brain abscess and UTIs (Sami *et al*., 2017). *Listeria* is a genus of a non-spore forming, non-encapsulated, rod shaped to coccoid facultative anaerobic Gram- and catalase positive bacteria that occur singly or in short chains. *Listeria* contains several species including *L. monocytogenes*, the only pathogenic species in human infections (Chen *et al*., 2017). Listeriosis in Africa has been scarcely reported in spite of the global increase in research cases. In Nigeria, information on *Listeria* in humans hardly exists. Little documentation has been from poor hygiene and cross-contamination of salad vegetables (cabbage/carrot/cucumber/lettuce/tomato), raw meat (chicken/pork/beef), meat and dairy products (chevon/cheese) and coleslaw (Dufailu *et al*., 2021).

In healthy non-pregnant women, asymptomatic bacteriuria may not necessitate any special attention. However, such bacteriuria in pregnancy requires special consideration because of the associated increased risk of maternal complications such as pyelonephritis and anaemia (Fatima and Mussaed, 2018; Edrees and Anbar, 2020). The presence of multidrug-resistant bacteria in pregnant HIV/AIDS patients is expected to increase the rate of therapy failures. Therefore, information resulting from this present study will provide a current baseline data on the predisposing effect of pregnancy and HIV infection on the growth of *Citrobacter* and *Listeria* species in pregnant women on antibiotic therapy.

## Materials and Methods

### *Study area*

The study was conducted among patients attending the Nigerian Air Force Hospital in Konjo, a satellite area of Makurdi, Benue State. The city lies between latitude $7^0 43^0$N, $07^0 45'$N and longitude $08^0 32'$E and $08^0 38'$E (Figure 1). It is located in the Middle Belt region of Nigeria along the Benue River experiencing yearly temperature fluctuations between $21^0$C and $37^0$C and both the wet (rainy) season that runs from April to October, and the dry season that begins in November and ends in March (Abah, 2012; Oyatayo *et al*., 2020). Based on a 2006 census, Makurdi, doubling as the state capital and headquarters of Makurdi Local Government Area, is the major city in Benue State and has a population of 297,398 with an average density of 323 people per square kilometre with the Tiv, Idoma and Igede speaking population predominating over others. Out of this total population, the number of males preponderates (157,295) that of the females (140,103) with a population increase in the next 30 years estimated at 142% (Shabu *et al*., 2021).

### *Institutional ethical approval*

Approval to undertake this study was sought from, and granted by the Project Research Committee of the Department of Microbiology Board of the Federal University Wukari and The Air force Base Hospital,

Makurdi. This research was then conducted in accordance with the Helsinki declaration.

***Study population and inclusion criteria***

Informed consent was sought from pregnant HIV outpatients of the hospital who were receiving antibiotics along with antiretrovirals at the time of the research. Those who consented and whose ages were above eighteen (18) years were enrolled into the study and constituted of eighty-two (82) females. Any patient that did not meet with any of these criteria was excluded from the study.

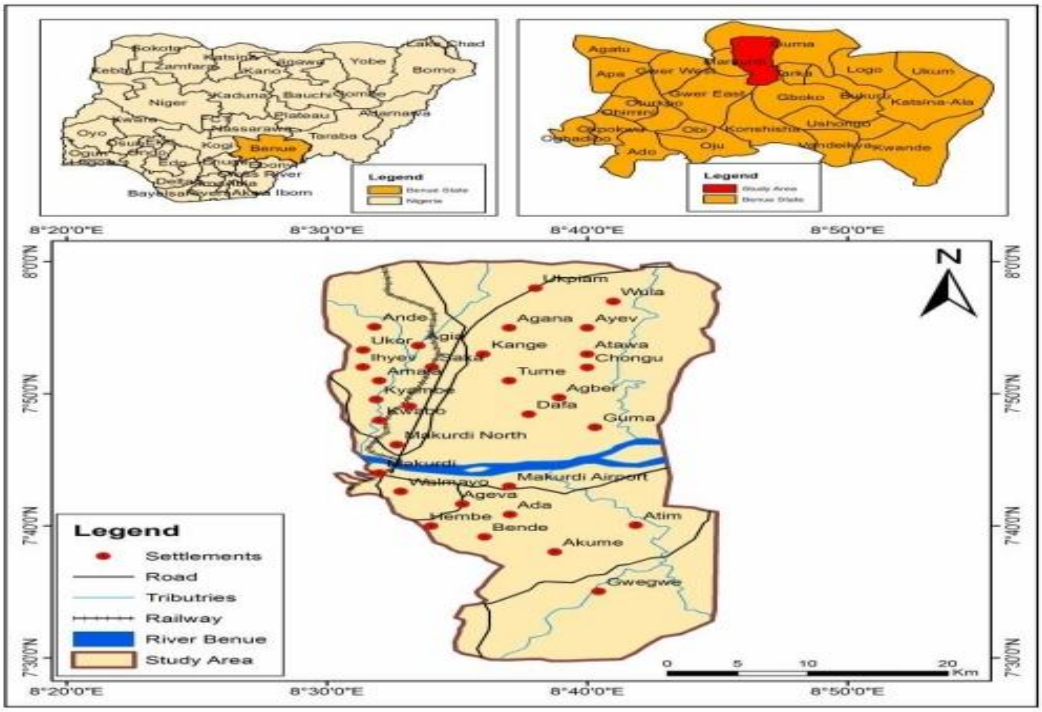


**Fig. 1: Administrative map of Benue State (Source: Adapted from Oyatayo *et al*., 2018).**

***Sample collection and cultivation***

A sterile screw capped universal container was given to each patient with basic instructions on how to collect clean catch midstream urine. Samples collected using this standard method were labelled and cultured on nutrient, cysteine lactose electrolyte deficient (CLED) and eosin methylene blue (EMB) agar growth to occur within an hour of collection, on agar plates and incubated at $37^0$C for a maximum of 24 hours. The media used were prepared from ready-to-use dehydrated agar powder according to their manufacturers' instructions (Cheesbrough, 2006).

***Bacterial identification and isolation***

After incubation, the growth colonies were macroscopically observed and the morphological characteristics recorded based on the general appearance (colour, size, shape, border, texture etc) of individual bacterial colonies on each plate. Thereafter, a single representative colony on each culture media plate was Gram stained and microscopically examined to determine the character and the arrangement of the bacterial cell (Cheesbrough, 2006).

Bacteria were isolated using the streak plating technique (Cheesbrough, 2006). An inoculum was picked from the 24 hour culture plate and a pool of each bacterial culture was aseptically made on a fresh nutrient agar plates. An inoculating wire loop was used to make streaks on the culture plates which were then incubated upside down position at a temperature of $37^0$C for 24 hours to obtain axenic cultures.

***Biochemical tests***

Following overnight incubation biochemical tests were carried out to properly characterize the bacterial isolates (Cheesbrough, 2006).

The catalase test was performed to demonstrate the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide. A small amount of culture to be tested from a nutrient agar slope was picked up using a sterile platinum loop and inserted into hydrogen peroxide solution held in a small tube. A positive test reaction was indicated by a bubbling of the mixture.

The dye, N,N,N′,N-Tetramethyl-p-phenylene-diamine dihydrochloride (TMPPD) is a redox mediator used for bacterial identification in a test known as the oxidase test which determines the presence in bacteria, of the oxidase enzyme that catalyses the transport of electrons between electron donors in the bacteria and the redox dye, TMPPD. A drop of 1% aqueous solution of TMPPD was added on to a piece of filter paper in a Petri dish. Subsequently, a smear of the pure culture was made on to the impregnated filter paper using a sterile platinum loop. A positive test reaction was recorded by observing for the formation of a blue-purple colouration after 5 minutes.

The coagulase test was used to demonstrate the ability of bacteria to produced coagulase, an enzyme that causes the clotting of blood plasma. This was carried out by homogenizing a loopful of the 24-hour pure culture in a drop of normal saline added on to a slide followed by the addition of a drop of human plasma to the suspension. This mixture was stirred for 5 minutes and a positive test

reaction recorded when a formation of a clot was observed.

***Antimicrobial susceptibility testing***

On the basis of their frequent usage in the study area, ten (10) groups of antibiotic discs (ciprofloxacin, ampicillin, cefalexin, erythromycin, ofloxacin, spiramycin, pefloxacin, trimethoprim and amoxicillin) were selected and used to determine the antibiogram of bacterial cultures. The antibiotic sensitivity testing (AST) was performed using the M100 standards recommended by the Clinical and Laboratory Standard Institute, CLSI. Inocula adjusted to 0.5 McFarland standard was swabbed on Mueller Hinton agar plates for antibiotic sensitivity assay (CLSI, 2022).

**Data analysis**

The percentage occurrence was calculated and recorded as percentages.

**Results and Discussion**

The phenotypic characteristics of *Citrobacter* and *Listeria* species are shown in Table 1. Biochemically, *Citrobacter* species was Gram negative, catalase and oxidase positive but coagulase negative while *Listeria* species was positive for all parameters investigated. *Citrobacter* are uncommon opportunistic commensals that cause urosepsis, meningitis, intra-abdominal abscesses, joint, blood stream, urinary tract and neonatal infections amidst other infections such as neonatal sepsis infection while *Listeria* spp. is known to usually have a low prevalence but high fatality rate and may cause varying clinical manifestations among persons. Mild infections in pregnant mothers can harm the foetus leading to preterm delivery, stillbirth, foetal death, or serious neonatal morbidity like septicaemia, pneumonia, meningitis and encephalitis (Welekidan *et al*., 2019; Dufailu *et al*., 2021).

**Table 1: Morphological characteristics of *Citrobacter* and *Listeria* species**

| **Colonial characteristics** | **Microscopy** Shape | **Biochemical tests** Gram reaction | Catalase | Oxidase | Coagulase | **Bacterial species** |
|---|---|---|---|---|---|---|
| Shiny, pink, round, smooth, tiny and slightly raised | Paired rods | - | + | + | - | *Citrobacter* |
| Light pink, round, smooth, tiny and raised | Single rods | + | + | + | + | *Listeria* |

Key: - = Negative + = Positive,

Table 2 shows that the overall prevalence of *Citrobacter* and *Listeria* was 31.7%. Both infections were higher (38.7%) in young adults that were between 18 and 30 years than in older adults (27.5%) that were above 50 years of age. No reason could be adduced for this as it is known to affect every individual equally irrespective of age

The parous-associated occurrence of *Listeria* and *Citrobacter* in the urine of females with HIV is highlighted in Table 3. Pregnant women harboured more of the two pathogens than non-pregnant females with the former having more (66.7%) of *Citrobacter* than *Listeria* species (33.3%). This is expected because since pregnancy is associated with reduced immunity, they are more susceptible to infectious agents with the already compromised immunity state caused by the virus (Abdul and Abbas, 2020).

In Table 4, *Citrobacter* spp. was resistant to all the tested antibiotics. While bulk of research in Africa reported the susceptibility of *Listeria* to amikacin, ampicillin, ciprofloxacin and gentamycin (Dufailu *et al*., 2021), this present result shows that it was susceptible to pefloxacin (13mm) and cefalexin (15mm) but resistant to all other antibiotics including ciprofloxacin and ampicillin.

**Table 2: Distribution of *Listeria* and *Citrobacter* species in young adults and the elderly**

| **Age** | **Number examined** | **Number infected** *Listeria* **spp.** | ***Citrobacter* spp.** | **Total** |
|---|---|---|---|---|
| **18-30** | **31** | **4(33.3)** | **8(66.7)** | **12(38.7)** |
| **>30** | **51** | **3(21.4)** | **11(78.6)** | **14(27.5)** |
| **Total** | **82** | **7(26.9)** | **19(73.1)** | **26(31.7)** |

**Table 3: Occurrence of *Listeria* and *Citrobacter* uropathogens in pregnant females with HIV**

| **Status** | **Number examined** | **Number infected** | **Bacterial isolate** *Listeria* spp. | *Citrobacter* spp. |
|---|---|---|---|---|
| Pregnant | 31 | 15(48.8) | 5(33.3) | 10(66.7) |
| Non pregnant | 51 | 11(21.6) | 2(18.2) | 9(81.8) |
| Total | 82 | 26(31.7) | 7(26.9) | 19(73.1) |

**Table 4: Anti-microbial susceptibilities of *Listeria* and *Citrobacter* species**

| **Bacterial spp.** | **CPX** | **SXT** | **E** | **PEF** | **CN** | **APX** | **AM** | **AU** | **OFX** | **SP** |
|---|---|---|---|---|---|---|---|---|---|---|
| *Listeria* | R | R | R | R | 13 | 15 | R | R | R | R |
| *Citrobacter* | R | R | R | R | R | R | R | R | R | R |

Key: CPX = Ciprofloxacin SXT = Trimethoprim E = Erythromycin PEF = Pefloxacin CN = Cefalexin APX = Amoxicillin

AM = Ampicillin AU = Augmentin OFX = Ofloxacin SP = Spiramycin

**Conclusions and recommendations**

Globally, drug-resistant bacterial infections are common with HIV patients (Rameshkumar and Arunagirinathan, 2018). In pregnancy, the introduction of antibiotic unresponsive *Citrobacter* and *Listeria* species will only add to the expenditure of healthcare interventions because the economic burden of antibiotic-not-susceptible isolates in uncomplicated urinary tract infection is enormous (Shafrin *et al*., 2022). Since this current study has shown the propensity of these bacteria to complicate therapy, it is advisable to carry out proper culture and sensitivity to antibiotics before the commencement of any antibiotic medication especially in pregnant women or if on empiric therapeutic use, allow for a change in the choice of antibiotics to forestall an increase in their epidemiology. In order to understand and mount effective prevention and control strategies and reveal population structure, dynamics of pathogen transition and transmission patterns, molecular studies especially genomic sequencing should be planned and researched on as soon as it is possible.

**Conflict of interest**

The authors declare no competing interests.

**References**


Abah RC 2012. Causes of seasonal flooding in floodplains: a case of Makurdi, Northern Nigeria. *Int. J. Env. Stud*., 69(6): 904-912.

Abdul K & Abbas MD 2020. Diseases of Immunity. In: *Robbins and Cotran Pathologic Basis of Diseases*. Kumar V. Abbas AK & Fausto N. eds. 10th ed. Philadelphia: Saunders. 245-258.

Barnie PA. Akwetey S. Swallah MH. Acheampong DO & Kwakye-Nuako G 2019. Occurrence and distribution of bacterial uropathogens among antiretroviral therapy users and non-users, Cape Coast Teaching Hospital. *Am. J. Multidiscipl. Res.*, 8(1): 543-549.

Chen JQ. Regan P. Laksanalamai P. Healey S & Hu Z 2017. Prevalence and methodologies for detection, characterization and sub typing of *Listeria monocytogenes* and *L. ivanovii* in foods and environmental sources. *Food Sci. and Hum. Wellbeing*, 6(3): 97-120.

Cheesbrough M 2006. *District Laboratory Practice in Tropical Countries*, Part 2, 2nd edn. Cambridge University Press Cape town, South Africa. ISBN 978-0-521-67632-8. 440p.

Clinical and Laboratory Standard Institute, CLSI 2022. *Performance Standards for Antimicrobial Susceptibility Testing*. M100. 32nd Edition. Clinical and Laboratory Standards Institute, Wayne, PA.

Dufailu OA. Yaqub MO. Owusu-Kwarteng J & Addy F 2021. Prevalence and characteristics of *Listeria* species from selected African countries. *Trop. Dis. Travel. Med. Vaccines*, *7*: 26

Edrees HW & Anbar AA 2020. Prevalence and antibacterial susceptibility of bacterial uropathogens isolated from pregnant women in Sana'a, Yemen. *Biol. Res.*, 5(4): 157-165.

Fatima SS & Mussaed EA 2018. Case Study. In: *Bacterial Identification and Drug Susceptibility Patterns in Pregnant and Non Pregnant UTI Patients*. *SpringerBriefs in Applied Sciences and Technology*. Singapore. ISBN 978-981-10-4750-3. Pp. 61-78.

Fonsah, JY. Njamnshi AK. Kouanfack C. Qiu F. Njamnshi DM. Tagny CT. Nchindap TE. Leopoldine K. Mbanya D. Heaton R & Kanmogne GD 2017. Adherence to antiretroviral therapy (ART) in Yaoundé-Cameroon: association with opportunistic infections, depression, ART regimen and side effects. *PLoS One*, 12(1): e0170893.

Niemogha MT. Umeana F. Awujo NC. Uwaezuoke JC. Onubogu OC & Odunukwe NN 2009. *In vitro* assessment of brands of Ampicillin sold in Nigerian markets. *Nig. J. Hlth. and Biomed. Sci.*, 8(1): 25-31.

Oyatayo KT. Ndabula C. Abaje IR. Jidauna GG. Iwan MT & Mshelia AM 2020. Trend analysis of rainfall and its implications for flooding in Makurdi drainage basin, Benue State, Nigeria. *FUDMA Int. J. Soc. Sci.*, 2(2): 41-58.

Rameshkumar MR & Arunagirinathan N 2018. Drug-resistant bacterial infections in HIV patients. *Adv. in HIV and AIDS Control*, 83-120.

Sami H. Sultan A. Rizvi M. Khan F. Ahmed, S. Shukla I & Khan HM 2017. *Citrobacter* as a uropathogen, its prevalence and antibiotics susceptibility pattern. *CHRISMED J. Hlth. Res.*, 4(1): 23.

Shabu T. Fate S & Ukula MK 2021. Impact of urbanization on agricultural land in Makurdi Local Government Area of Benue State, Nigeria. NASS. J. Agr. Sci., 3(1): 1-8.

Shafrin J. Marijam A. Joshi AV. Mitrani-Gold FS. Everson K. Tuly R. & Ruiz ME 2022. Economic burden of antibiotic-not-susceptible isolates in uncomplicated urinary tract infection: analysis of a US integrated delivery network database. *Antimicrob. Resist. and Infect. Contr.*, 11(1): 1-13.

Welekidan IN. Bahta YW. Teklehaimanot MG. Abay GK. Wasihun AG & Dejene TA et al. 2019. Prevalence and drug resistance pattern of *Listeria monocytgenes* among pregnant women in Tigray region, northern Ethiopia: a cross-sectional study. *BMC Res. Notes*, 12(1): 1-6.