

**MOLECULAR CHARACTERIZATION OF *ENTEROBACTERIACEAE* FROM
PAEDIATRIC PATIENTS IN ABUJA AND KANO**

BY

DURU, CARISSA IJEOMA

2012667006F

**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES
NNAMDI AZIKIWE UNIVERSITY, AWKA;**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A
DOCTOR OF PHILOSOPHY (Ph.D) DEGREE IN PHARMACEUTICAL
MICROBIOLOGY AND BIOTECHNOLOGY**

**SUPERVISOR: PROFESSOR C.O. ESIMONE
SUPERVISOR: DR. E.C. NWANEGBO**

OCTOBER, 2018

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OCTOBER, 2018

CERTIFICATION

I, **Duru, Carissa Ijeoma**, a doctoral student in the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka with the Registration Number: **2012667006F** do hereby certify that the work embodied in this research work is original and has not been submitted in part or in full for any other Diploma or Degree in this or any other University or College.

The literature consulted during the course of the work has been duly acknowledged by means of appropriate references.

.....

Duru, Carissa Ijeoma

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APPROVAL PAGE

The title “Molecular Characterization of *Enterobacteriaceae* from Pediatric Patients in Central and North Western Nigeria” has been approved for **DURU, CARISSA IJEOMA**, a doctoral student, with Registration Number: **2012667006F**, of the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, NnamdiAzikiwe University, Awka.

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DEDICATION

The embodiment of this **dissertation** is dedicated solely to God Almighty who in His infinite mercies gave me good health to stand out in my academic pursuit.

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At the end of the expedition of accomplishing my PhD, one of the joys of completion is to look over the journey past and remember all the people who have helped, supported and encouraged me along this long but fulfilling road.

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ABBREVIATIONS USED IN THIS DISSERTATION

GENERAL ABBREVIATIONS

ESBLs	Extended spectrum beta-lactamases
MDRGNOs	Multidrug resistant Gram negative organisms
ASM	American Society of Microbiology
CLSI	Clinical Laboratory Standard Institute
PBPs	Penicillin-binding proteins
AST	Antimicrobial susceptibility testing
IMP	Imipenemase
OM	Outer membrane
PBPs	Penicillin-binding proteins
NAM	N-acetylmuramic acid
NAG	N-acetyl glucosamine
SME	<i>Serratia marcescens</i> Carbapenemases
GES	Guiana extended spectrum
EDTA	Ethylene diamine tetraacetic acid
SPM	Sao Paulo metallo-beta-lactamase
GIM	German-imipenemase metallo-beta-lactamase
OXA	Oxacillin
NDM	New Delhi Metallo-beta-lactamase
ICUs	Intensive care units
HDUs	High-dependency units
DNA	Deoxyribonucleic acid
CLSI	Clinical Laboratory Standard Institute

MH	Mueller Hinton
IPM	Imipenem
MEM	Meropenem
FOX	Cefoxitin
CAZ	Ceftazidime
AK	Amikacin
CN	Gentamicin
CTX	Cefotaxime
CRO	Ceftriaxone
CIP	Ciprofloxacin
OX	Oxacillin
ATM	Aztreonam
ATCC	American Type Culture Collection
E	Epsilometer
MR	Methyl red test
VP	Voges proskauer
S	Susceptible
I	Intermediate
R	Resistant
PCR	Polymerase chain reaction
Bla:	β -Lactamase coding gene
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool

CLSI	Clinical and Laboratory Standards Institute
CTX-M	Active on Cefotaxime, First Isolated at Munich
DNA	Deoxyribonucleic acid
dNTP	deoxynucleotide triphosphates
FASTA format	text-based format for representing either nucleotide Sequences or peptide sequences
KPC	<i>Klebsiella pneumoniae</i> carbapenemase
NCBI	National Center for Biotechnology Information
SHV	Sulphydryl Reagent Variable
ST	Sequence Type
TEM	Named after Patient Temoniera

ABSTRACT

Bacteremia is a leading cause of death in developing countries but etiologic evaluation is infrequent and empiric antibiotics are not evidence-based. Very little is known about the types of extended-spectrum β -lactamases (ESBL) in pediatric bacteremia patients in Nigeria. We evaluated the prevalence of ESBL in *Enterobacteriaceae* and their patterns of antibiotic resistance in children enrolled into surveillance for community acquired bacteremic syndromes across health facilities in Central and North West-Nigeria. Blood culture from suspected cases of sepsis from children of age less than 5 years were processed using automated Bactec® incubator System from Sept 2008-Dec 2016. *Enterobacteriaceae* bacteria were identified to the species level using Analytical Profile Index (API20E®) identification strip and antibiotic susceptibility profile was determined by the disc diffusion method. The multidrug resistant strains were then screened and confirmed for extended spectrum beta lactamase (ESBL) production by the combination disc. Real time polymerase chain reaction was used to elucidate the genes responsible for ESBL production. The complete genome of 16 carbapenem resistant ESBL isolates was obtained via Next Seq whole-genome sequencing. A total of 413 *Enterobacteriaceae* were screened for ESBL. Of these, 38.74% produced ESBL. Of the ESBL organisms, 65.62% were *Klebsiella pneumoniae*, 13.12% were *Enterobacter cloacae*, 13.75% were *Escherichia coli*, 2.5% were *Serratia species*, 4.37% were *Pantoea species* and 0.62% were *Citrobacter species*. High resistance rates were observed among ESBL-positive isolates for ceftriaxone (92.3%), aztreonam (96.8%), cefpodoxime (96.25%), cefotaxime (98.75%) and sulphamethoxazole-trimethoprim (90%), while 87.5%, 90.63%, and 91.87% of the isolates were susceptible to imipenem, amikacin and meropenem respectively. Frequently detected resistance genes were *bla*TEM 83.75% and *bla*CTX-M 83.12% followed by *bla*SHV genes 66.25%. Sequencing-based analyses revealed a prevalence of 25% New Delhi metallo-beta-lactamase (NDM) -5- producing ST476 and 6.25% oxacillinase (OXA) -48- producing ST 1788 *Klebsiella pneumoniae* isolates. Most of the isolates possessed plasmid replicons; IncF and IncHI2. The plasmid multilocus sequence type (pMLST) showed that IncFII exhibited 3 pMLST as K7:A-:B- K13:A-:B- and K8:A-:B- .

In conclusion, our results indicate a high rate of ESBL resistance to commonly used antibiotics in *Enterobacteriaceae* bacteremia in children from Central and North-West Nigeria.

Salmonella typhi

100

0(0)

100 (39.52)





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ETHICS APPROVAL

Further to your request for extension in respect of your research proposal titled "Community Acquired Pneumonia and Invasive Bacteraemic Diseases (CAPIBD)", the Committee reviewed your progress report and noted same.

In view of the above, Ethics approval is hereby granted to continue with the research till December 2015.

However, the approval is subject to periodic reporting of the progress of the study and its completion to the Research Ethics Committee.

Regards

Abubakar S. Mahmud
Secretary, Research Ethics Committee
For: Chairman