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1    **Phenotypic and genotypic characterization of clinical carbapenems resistant**  
2    ***Enterobacteriaceae* isolates from Sokoto, northwest Nigeria**

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22    **Running title:** Phenotypic and Genotypic characterization of carbapenemase-producing  
23    *Enterobacteriaceae*.

## Abstract:

Emergence and spread of carbapenemase producing *Enterobacteriaceae* (CPE) is one of the major problems currently threatening the global public health. In Nigeria, interest on CPE begins recently. In Sokoto, northwest Nigeria, there is no data on the prevalence and mechanism underlying carbapenem resistance. In this study, we aimed to investigate the presence of clinical carbapenems resistant *Enterobacteriaceae* isolates in two leading hospitals in Sokoto, Northwest-Nigeria. A total of 292 non-duplicate *Enterobacteriaceae* isolated from clinical specimens processed in the diagnostic laboratories of the two hospitals between January and June 2019 were collected. Of these, 129 (44.2 %) and 19 (6.5%) were resistant to third generation cephalosporin (3CG) and carbapenems, respectively. The result of RT-PCR revealed that 10 (7.8 %), 19 (14.7 %) and 46 (35.7 %) of the 3GC resistant isolates harboured *bla<sub>SHV</sub>*, *bla<sub>TEM</sub>* and *bla<sub>CTX-M</sub>* genes, respectively. The modified Carba NP test result showed that only 7 (36.8 %) of the 19 carbapenems-resistant isolates were carbapenemase producing; among them *bla<sub>NDM-5</sub>* and *bla<sub>OXA-181</sub>* genes were identified in five and two isolates, respectively. However, none of the investigated carbapenemase genes including *bla<sub>VIM</sub>*, *bla<sub>KPC</sub>*, and *bla<sub>IMP</sub>* was detected in the remain carbapenems-resistant isolates suggesting therefore a non-enzymatic mechanism. This study reports for the first time, the emergence of CPE in Sokoto state and first detection of NDM-producing *Citrobacter freundii* in Nigeria. The observed CPE in this study is concerning in a country where alternative antibiotics is rarely available.

**Keywords:** Carbapenem resistance, Carbapenemase genes, *bla<sub>NDM-5</sub>*, *bla<sub>OXA-181</sub>*, Nigeria

## 22    **Introduction**

23            Carbapenems are highly effective  $\beta$ -lactam antibiotics introduced into clinical practices  
24 following the emergence of plasmids encoding for extended-spectrum  $\beta$ -lactamases [1]. The  
25 carbapenems are among the last resort armamentarium against infections due to multidrug  
26 resistant (MDR) Gram-negative bacteria [2]. Until the early 1990s, resistance to carbapenems has  
27 been mostly due to chromosomal  $\beta$ -lactamases [3]. The transferable plasmid encoded  
28 carbapenemase (*bla*<sub>IMP-1</sub>) emerged in Japan in 1990 [4]. Following this, there was rapid  
29 dissemination of carbapenemase producing bacteria and it continues to be increasingly reported  
30 worldwide [5]. Other recognised carbapenem resistance mechanisms include decreased outer  
31 membrane permeability and up-regulation of efflux system with/without production of extended-  
32 spectrum  $\beta$ -lactamases (ESBLs) [6]. However, acquired carbapenemase production is the most  
33 clinically important carbapenem resistant mechanism [5].

34            The emergence of carbapenemases-producing *Enterobacteriaceae* (CPE) is concerning, as  
35 it further limits option for treatment of infections due to MDR Gram negative bacteria [7]. It has  
36 been described as an imminent threat to global public health with attendant morbidity and  
37 mortality [8–10]. Annually, carbapenem resistant Gram-negative bacteria (CR-GNB) cause  
38 approximately 9’300 infections in the United States, half of which usually result in death [11].  
39 Moreover, longer duration of hospital stays and consequently increased healthcare cost is  
40 associated with CR-GNB infections [12,13]. In the United States, *bla*<sub>KPC</sub> is the most common  
41 carbapenemase genes, though recent emergence of *bla*<sub>NDM</sub> producing *Enterobacteriaceae* has been  
42 reported particularly among patients returning from *bla*<sub>NDM</sub> endemic region [14]. Moreover,  
43 despite that wide geographical variation exists in the types of carbapenemase genes in Europe,  
44 generally *bla*<sub>KPC</sub> and *bla*<sub>OXA-48</sub> are the commonest carbapenemase enzymes [15,16]. In Nigeria,  
45 there is dearth of data on the genetic diversity and spread of CPE. Interest in research on  
46 carbapenem resistance begins recently. Majority of studies on CPE have been limited to

phenotypic testing [17,18]. However, a few studies have used genotypic methods to establish the occurrence of carbapenemase genes among clinical and non-clinical bacterial isolates in Nigeria [19,20]. In Sokoto however, there was no data on the prevalence of CPE. In view of this, we therefore aimed to investigate the prevalence of clinical *Enterobacteriaceae* isolates bearing carbapenemase genes in two-leading hospitals in Sokoto, Northwest-Nigeria and also to characterise their molecular resistance mechanisms.

## **Materials and methods**

### **Study area, design and period**

This was a prospective, descriptive and epidemiological study conducted between January and July 2019 among patients attending the two main tertiary healthcare facilities in the capital of Sokoto state, Sokoto, Northwest Nigeria. The hospitals are the largest hospitals located within the state. The hospitals, Usman Danfodiyo University Teaching Hospital (UDUTH) and Sokoto State Specialist Hospital (SHS), are respectively 850 and 300-beds hospitals and rendering essential, specialized and referral medical and surgical services to residents of Sokoto state and patients from adjoining states of Zamfara, Kebbi and Niger within Nigeria and also to referral cases from the neighbouring Niger Republic.

### **Bacterial collection and identification**

Non-duplicate clinical *Enterobacteriaceae* isolates were collected from the pool of biological specimens submitted and processed by the diagnostic microbiological laboratories of the two hospitals. The isolates were preliminarily identified by a combination of morphology and conventional biochemical tests for *Enterobacteriaceae* using the standard microbiological techniques. The isolates were then preserved on nutrient agar slants and subsequently shipped to Microbial Evolution and Phylogeny Infection, Institute Hospital University, Marseille in France for further characterisation. The identity of the isolates was confirmed using matrix-assisted laser

desorption ionisation time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany) according to the protocol previously described [21].

### **Antibiotic Susceptibility Test**

Antibiotic susceptibility test (AST) was carried out using the modified Kirby-Bauer disc diffusion method as outlined in the current European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, Version 9.0, 2019 [22]. The test was performed on Mueller–Hinton agar plates using the following commercially available discs (Oxoid limited, UK): carbapenems (imipenem and ertapenem); cephalosporins (ceftriaxone, cefalotine, and cefepime); fluoroquinolones (ciprofloxacin); aminoglycosides (amikacin and gentamicin); tetracycline (doxycycline). Others are trimethoprim-sulfamethoxazole, amoxicillin-clavulanate, fosfomycin and nitrofurantoin. The AST results were interpreted according to EUCAST breakpoints. The imipenem and ertapenem minimum inhibitory concentrations (MIC) for isolates with reduced susceptibility to either imipenem or/and ertapenem by disc diffusion test was thereafter determined using the gradient diffusion tests (Etest®, bioMérieux, Marcy L'Etoile, France). Any of the *Enterobacteriaceae* isolates that exhibits resistance to either of the carbapenem antibiotics (imipenem or ertapenem) would be regarded as carbapenem resistant as defined by the Centres for Disease Control and Prevention (CDC) [23]. The definition however requires reduced susceptibility to carbapenems other than imipenem for the trio of *Proteus* spp., *Morganella morganii* and *Providencia* spp. [23].

### **Phenotypic detection of ESBL and carbapenemase enzymes production**

The phenotypic detection of ESBL enzymes was performed using the double-disk synergy test by placing a  $\beta$ -lactamase inhibitor (amoxicillin-clavulanic and piperacillin-tazobactam) discs between two third generation cephalosporins (3GCs) at a distance of 20 mm centre-to-centre.[24] Formation of a characteristic keyhole effect or champagne-cork shaped zone of inhibition between the discs was considered as a phenotypic indication of ESBL production. The carbapenem

resistant isolates were screened for phenotypic carbapenemase production using the modified Carba NP test as previously described [25].

## **Molecular characterization of ESBL and carbapenemase genes**

The 3GC and carbapenem resistant isolates were screened for the presence of genes encoding ESBLs (*bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>) and carbapenemases (*bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>NDM</sub> and *bla*<sub>KPC</sub>), using the quantitative real-time PCR (qPCR) as previously described [26]. The qPCR positive isolates were confirmed by conventional PCR. The genetic variant of the carbapenemase genes was determined by sequencing of the positive PCR amplicons in both directions using the same set of standard PCR primers with BigDye Terminator on an automated ABI 3500XL genetic analyser (Applied Biosystems, Foster City, CA) according to the previously protocol described [27]. The generated raw read sequences were assembled using codon code aligner, v 9.0.1 (Codon Code Corp., Massachusetts, USA). The assembled sequences were identified by Blast analysis against the ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation) database [28].

## **Genotypic investigation of colistin resistance mechanism**

Moreover, one of the leading objectives of this work was to investigate colistin resistance in these clinical isolates. Irrespective of the results of phenotypic colistin resistance test, qPCR was used to screen the whole collection of the 292 *Enterobacteriaceae* isolates for mobilized colistin resistance (*mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5* and *mcr-8*) genes, as previously described [29].

## **Result**

The distribution of the clinical isolates recovered during clinical diagnostic testing in the two hospitals shows that majority of the isolates were recovered from urine 143 (49.0 %) and stool

119 76 (26.0 %) specimens. Others were obtained from sputum 21 (7.2 %), wound swab 29 (9.9 %)  
120 and ear swab 6 (2.1 %).

121 Out of the 292 *Enterobacteriaceae* isolated from the two hospitals during the study period,  
122 129 (44.2 %) and 19 (6.5%) were resistant to third generation cephalosporin (3GC) and  
123 carbapenems, respectively. The distribution of the isolates is presented in Table 1. The third  
124 generation cephalosporins (3GC) resistant comprises *C. freundii* (n=10), *E. cloacae* (n=18), *E. coli*  
125 (n=51), *K. pneumoniae* (n=28), *M. morganii* (n=4), *Proteus mirabilis* (n=14), *Providencia rettgeri*  
126 (n=1) and *Providencia stuartii* (n=3). The phenotypic ESBL screening result showed that 36 of the  
127 3GC resistant bacteria are ESBL positive. The result of RT-PCR revealed that 10 (7.8 %), 19 (14.7  
128 %) and 46 (35.7 %) of the 3GC resistant isolates harboured *bla<sub>SHV</sub>*, *bla<sub>TEM</sub>* and *bla<sub>CTX-M</sub>* genes,  
129 respectively (Table 1). While 9 and 8 of the isolates co-harboured *bla<sub>TEM</sub>* and *bla<sub>CTX-M</sub>* and *bla<sub>SHV</sub>*  
130 and *bla<sub>CTX-M</sub>*, respectively, one of the isolates co-expressed the three ESBL genes.

131 The carbapenem resistant *Enterobacteriaceae* (CRE) on the other hand includes  
132 *Citrobacter freundii* (n=3), *Enterobacter cloacae* (n=6), *Escherichia coli* (n=8) and *Klebsiella*  
133 *pneumoniae* (n=2). The result of modified CarbaNP test however showed positive result for only 7  
134 (36.8 %) of 19 CRE suggestive of carbapenemase expression in these isolates. As presented in  
135 Table 2, PCR and sequencing results revealed that the seven carbapenemase-producing strains  
136 harboured *bla<sub>NDM-5</sub>* (n=5) and *bla<sub>OXA-181</sub>* (n=2). However, 12 of the CRE isolates did not harbour  
137 any of the investigated carbapenemase genes suggestive of a non-enzymatic resistance mechanism  
138 in these isolates. Thus, we planned in perspective to investigate the carbapenems resistance  
139 mechanism in these isolates by whole genome sequencing (WGS) approach.

140 The result of antibiotic susceptibility testing revealed that, with the exception of the  
141 naturally colistin resistant strains of *Proteus*, *Morganella*, *Serratia* and *Providencia* isolates, none  
142 of the isolates was resistant to colistin. Molecular detection by PCR targeting six *mcr* gene  
143 variants was negative for all the 292 collected isolates in this study.



## Discussion

Globally, carbapenem resistance is increasingly reported [30]. The prevalence of which varies from one geographical region to the other. In the present study, the overall prevalence 6.5 % carbapenem resistance was reported. Previous reports across the country have established varying rates. For example, 28 % carbapenem resistance rate was reported in the preceding year in one of the hospitals among carbapenem naïve patients [18]. Also, in a neighbouring west Africa country, Ghana, 66% CR-GNB rate has been reported [31]. Despite the poor drug regulatory system in Nigeria coupled with the lack of an established antibiotic stewardship, carbapenem use in both hospital and community is generally low, reserved as a last resort agent against life threatening infections by multidrug resistant bacteria [32]. The observed resistance to the carbapenems in this study is troubling in a country where alternative antibiotics is rarely available [32]. Furthermore, the emergence and spread of carbapenem resistance bacteria is more worrisome because of lack of laboratory capacity for its detection [32]. The emergence and dissemination of CRE in the present study may be attributed to a number of factors. The carbapenem resistance may have emerged independently as a result of selection pressure of overuse of  $\beta$ -lactam antibiotics [33]. Beta-lactam antibiotics are the most widely used, often inappropriately, antibiotics in both community and hospital settings in Nigeria [34]. In addition, the CRE could have been imported by patients returning from international travelling to regions like India and Europe where carbapenem resistance is endemic [35,36]. Reports of importation of antibiotic resistant bacteria across geographical border has been documented [37].

In this study, the presence of *bla*<sub>NDM-5</sub> and *bla*<sub>OXA-181</sub> accounted for carbapenem resistance in about a third of the CRE isolates. This corresponds to the findings of previous studies in different regions of Nigeria where *bla*<sub>NDM-5</sub>, *bla*<sub>OXA-48</sub> and *bla*<sub>OXA-181</sub> have been reported as the commonest carbapenemase genes [38,39]. Our findings however contrasted the report of a study in Maiduguri, northeast-Nigeria, where *bla*<sub>KPC</sub> has been reported as the predominant

169 carbapenemase gene [40]. Other mechanisms such as ESBLs and/or plasmid AmpC enzyme  
170 production with reduced outer membrane permeability may be responsible for the carbapenem  
171 resistance in the remaining isolates [41].

172 While we did not find any isolates bearing *mcr* genes in this study, reports of clinical  
173 isolates from Nigeria harbouring plasmid encoding colistin resistance genes have begun to surface  
174 in the literatures.[42,43] Our finding however concurs with other studies where clinical bacterial  
175 isolates have been documented to be highly susceptible to colistin.[44,45]

176 This study is the first to comprehensively investigate molecular basis of resistance to  
177 carbapenems in northwest-Nigeria. The diversity of the strains investigated adds to the robustness  
178 of the study as previous studies concentrated on *K. pneumoniae* and *E. coli*. This permits the first  
179 detection of *Enterobacter cloacae* expressing NDM-carbapenemase in northwest-Nigeria and first  
180 description of carbapenemase producing *Citrobacter freundii* in Nigeria.

## 181 **Conclusion:**

182 Here, for the first time, we describe the emergence of CPE in Sokoto state and the first  
183 detection of NDM-producing *Citrobacter freundii* in Nigeria. The finding of this study is  
184 concerning in a country where alternative antibiotic is rarely available.

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193            The authors declare that they have no competing interests.

194    **Authors' contributions:**

195    This study was designed by YKEI, BOO, JMR, and SMD. The experiment was conducted by AO  
196    and LZN. AO drafted the first manuscript which was revised by all authors. All authors have read  
197    and approved the final manuscript.

198

- 200 [1] Iovleva A, Doi Y. Carbapenem-resistant *Enterobacteriaceae*. Clin Lab Med 2017;37:303–15.  
201 <https://doi.org/10.1016/j.cll.2017.01.005>.
- 202 [2] Nicolau DP. Carbapenems: a potent class of antibiotics. Expert Opin Pharmacother 2008;9:23–  
203 38.
- 204 [3] Queenan AM, Bush K. Carbapenemases: the Versatile beta-Lactamases. Clin Microbiol Rev  
205 2007;20:440–58. <https://doi.org/10.1128/CMR.00001-07>.
- 206 [4] Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable Imipenem Resistance in  
207 *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1991;35:147–51.
- 208 [5] Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA, et al. Carbapenemase-  
209 Producing Organisms: A Global Scourge. Clin Infect Dis 2018;66:1290–7.  
210 <https://doi.org/10.1093/cid/cix893>.
- 211 [6] Patel G, Bonomo RA. “Stormy waters ahead”: global emergence of carbapenemases. Front  
212 Microbiol 2013;4:1–17. <https://doi.org/10.3389/fmicb.2013.00048>.
- 213 [7] Falagas ME, Tansarli GS, Karageorgopoulos DE. Deaths Attributable to *Enterobacteriaceae*  
214 Infections. Emerg Infect Dis 2014;20:1170–5.
- 215 [8] Matsunaga N, Hayakawa K. Estimating the impact of antimicrobial resistance. Lancet Glob Heal  
216 2018;6:e934–5. [https://doi.org/10.1016/S2214-109X\(18\)30325-5](https://doi.org/10.1016/S2214-109X(18)30325-5).
- 217 [9] Founou RC, Founou LL, Essack SY. Clinical and economic impact of antibiotic resistance in  
218 developing countries : A systematic review and meta-analysis. PLoS One 2017;12:1–18.
- 219 [10] Judd WR, Ratliff PD, Hickson RP, Mt DMS, Kennedy CA. Clinical and economic impact of  
220 meropenem resistance in *Pseudomonas aeruginosa*-infected patients. Am J Infect Control  
221 2016;1–5. <https://doi.org/10.1016/j.ajic.2016.04.218>.
- 222 [11] CDC. Antibiotic resistance threats in the United States. 2013.
- 223 [12] Stewardson AJ, Marimuthu K, Sengupta S, Allignol A, El-bouseary M, Carvalho MJ, et al. Effect  
224 of carbapenem resistance on outcomes of bloodstream infection caused by *Enterobacteriaceae* in  
225 low-income and middle-income countries ( PANORAMA ): a multinational prospective cohort  
226 study. Lancet Infect Dis 2019;19:601–10. [https://doi.org/10.1016/S1473-3099\(18\)30792-8](https://doi.org/10.1016/S1473-3099(18)30792-8).
- 227 [13] Magiorakos AP, Burns K, Baño JR, Borg M, Daikos G, Dumpis U, et al. Infection prevention and  
228 control measures and tools for the prevention of entry of carbapenem-resistant  
229 *Enterobacteriaceae* into healthcare settings : guidance from the European Centre for Disease  
230 Prevention and Control. Antimicrob Resist Infect Control 2017;6:1–17.  
231 <https://doi.org/10.1186/s13756-017-0259-z>.
- 232 [14] Logan LK, Weinstein RA. The Epidemiology of Carbapenem-Resistant *Enterobacteriaceae*: The  
233 Impact and Evolution of a Global Menace. J Infect Dis 2017;215:1–9.  
234 <https://doi.org/10.1093/infdis/jiw282>.
- 235 [15] Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL. Carbapenemase-producing  
236 *Enterobacteriaceae* in Europe : assessment by national experts from 38 countries , May 2015.  
237 Eurosurveillance 2015;20:1–8.

- 238 [16] Dortet L, Cuzon G, Ponties V, Nordmann P. Trends in carbapenemase-producing  
239 *Enterobacteriaceae*, France, 2012 to 2014. *Eurosurveillance* 2017;22:1–9.  
240 <https://doi.org/10.2807/1560-7917.ES.2017.22.6.30461>.
- 241 [17] Alaka O, Orimolade E, Ojo O, Onipede A. The Phenotypic Detection of Carbapenem Resistant  
242 Organisms in Orthopaedic Wound Infections in Ile-Ife , Nigeria. *Acta Sci Microbiol* 22  
243 2019;2:35–42.
- 244 [18] Olowo-okere A, Abdullahi MA, Ladidi BK, Suleiman S, Tanko N, Ungokore HY, et al.  
245 Emergence of Metallo-B-Lactamase Producing Gram-Negative Bacteria In A Hospital With No  
246 History Of Carbapenem Usage In Northwest Nigeria. *Ife J Sci* 2019;21:323–31.
- 247 [19] Jesumirhewe C, Springer B, Lepuschitz S, Allerberger F, Ruppitsch W. Carbapenemase-  
248 Producing *Enterobacteriaceae* Isolates from Edo State, Nigeria. *Antimicrob Agents Chemother*  
249 2017;18:1–5. <https://doi.org/10.1128/AAC.00255-17>.
- 250 [20] Brinkac LM, White R, Nguyen K, Obaro SK, Fouts DE. Emergence of New Delhi Metallo- beta-  
251 Lactamase (NDM-5) in a Nigerian Hospital. *MSphere* 2019;4:1–10.
- 252 [21] Seng P, Drancourt M, Scola B La, Fournier P, Rolain JM, Raoult D. Ongoing Revolution in  
253 Bacteriology : Routine Identification of Bacteria by Matrix-Assisted Laser Desorption Ionization  
254 Time-of-Flight Mass Spectrometry. *Clin Infect Dis* 2009;49:543–51.  
255 <https://doi.org/10.1086/600885>.
- 256 [22] EUCAST. EUCAST guidelines for detection of resistance mechanisms and specific resistances of  
257 clinical and / or epidemiological importance. 2019.
- 258 [23] National Center for Emerging and Zoonotic Infectious Diseases. Facility Guidance for Control of  
259 Carbapenem-resistant *Enterobacteriaceae* ( CRE ) November 2015 Update 2015.  
260 <https://www.cdc.gov/hai/organisms/cre/cre-facilities.html> (accessed January 12, 2020).
- 261 [24] EUCAST. EUCAST guidelines for detection of resistance mechanisms and specific resistances of  
262 clinical and / or epidemiological importance. 2017.
- 263 [25] Bakour S, Garcia V, Loucif L, Brunel JM, Gharout-Sait A, Touati A, et al. Rapid identification of  
264 carbapenemase-producing *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter*  
265 *baumannii* using a modified Carba NP test. *New Microbes New Infect* 2015;7:89–93.  
266 <https://doi.org/10.1016/j.nmni.2015.07.001>.
- 267 [26] Lalaoui R, Bakour S, Livnat K, Assous MV, Diene SM, Rolain JM. Spread of Carbapenem and  
268 Colistin-Resistant *Klebsiella pneumoniae* ST512 Clinical Isolates in Israel: A Cause for  
269 Vigilance. *Microb Drug Resist* 2018;25:63–71. <https://doi.org/10.1089/mdr.2018.0014>.
- 270 [27] Mellouk FZ, Bakour S, Meradji S, Al-Bayssari C, Bentakouk MC, Zouyed F, et al. First Detection  
271 of VIM-4-Producing. *Microb Drug Resist* 2016;00:1–15. <https://doi.org/10.1089/mdr.2016.0032>.
- 272 [28] Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, et al. ARG-  
273 annot, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes.  
274 *Antimicrob Agents Chemother* 2014;58:212–20. <https://doi.org/10.1128/AAC.01310-13>.
- 275 [29] Nabti LZ, Sahli F, Ngaiganam EP, Radji N, Mezaghcha W, Lupande-mwenebitu D, et al.  
276 Development of real-time PCR assay allowed describing the first clinical *Klebsiella pneumoniae*  
277 isolate harboring plasmid-mediated colistin resistance mcr-8 gene in Algeria. *J Glob Antimicrob*  
278 *Resist* 2019. <https://doi.org/10.1016/j.jgar.2019.08.018>.

- 279 [30] Jamal WY, Albert MJ, Rotimi VO. High Prevalence of New Delhi Metallo-  $\beta$  - Lactamase-1 (   
280 NDM-1 ) Producers among Carbapenem-Resistant *Enterobacteriaceae* in Kuwait 2016;1:1–12.   
281 <https://doi.org/10.1371/journal.pone.0152638>.
- 282 [31] Codjoe FS, Donkor ES, Smith TJ, Miller K. Phenotypic and Genotypic Characterization of   
283 Carbapenem-Resistant Gram-Negative Bacilli Pathogens from Hospitals in Ghana 1,2. Microb   
284 Drug Resist 2019;00. <https://doi.org/10.1089/mdr.2018.0278>.
- 285 [32] NCDC. Antimicrobial Use and Resistance in Nigeria. 2017.
- 286 [33] Bush K. Past and Present Perspectives on beta-lactamases. Antimicrob Agents Chemother   
287 2018;62:1–20.
- 288 [34] Adisa R, Orherhe OM, Fakeye TO. Evaluation of antibiotic prescriptions and use in under-five   
289 children in Ibadan , SouthWestern Nigeria. Afr Health Sci 2018;18:1189–201.
- 290 [35] Brisse S, Doumith M, Woodford N, Hopkins KL, Aasnæs B, Sundsfjord A, et al. Molecular and   
291 epidemiological characterization of carbapenemase- producing *Enterobacteriaceae* in Norway ,   
292 2007 to 2014. PLoS One 2017;12:1–17.
- 293 [36] Duin D Van, Doi Y. The global epidemiology of carbapenemase- producing *Enterobacteriaceae*.   
294 Virulence 2017;8:460–9. <https://doi.org/10.1080/21505594.2016.1222343>.
- 295 [37] Okeke IN, Edelman R. Dissemination of Antibiotic-Resistant Bacteria across Geographic   
296 Borders. Clin Inf Dis 2001;33:364–9.
- 297 [38] Shettima SA, Tickler IA, Cruz CM, Tenover FC. Characterization of Carbapenem-Resistant   
298 Gram-Negative Organisms from Clinical Specimens in Yola, Nigeria. J Glob Antimicrob Resist   
299 2019. <https://doi.org/10.1016/j.jgar.2019.08.017>.
- 300 [39] Olalekan A, Onwugamba F, Iwalokun B, Mellmann A, Becker K, Schaumburg F. High   
301 proportion of carbapenemase producing *Escherichia coli* and *Klebsiella pneumoniae* among   
302 extended spectrum beta-lactamase producers in Nigerian hospitals. J Glob Antimicrob Resist   
303 2019. <https://doi.org/10.1016/j.jgar.2019.09.007>.
- 304 [40] Mohammed Y, Zailani SB, Onipede AO. Characterization of KPC , NDM and VIM Type   
305 Carbapenem Resistance *Enterobacteriaceae* from North Eastern , Nigeria. J Biosci Med   
306 2015;3:100–7.
- 307 [41] Ye Y, Xu L, Han Y, Chen Z, Liu C, Ming L. Mechanism for carbapenem resistance of clinical   
308 *enterobacteriaceae* isolates. Exp Ther Med 2018;15:1143–9.   
309 <https://doi.org/10.3892/etm.2017.5485>.
- 310 [42] Otokunefor, K; Tamunokuro, E; Amadi A. Molecular Detection of Mobilized Colistin Resistance   
311 ( mcr-1 ) gene in *Escherichia coli* Isolates from Port Harcourt , Nigeria. J Aplplied Environ   
312 Manag 2019;23:401–5.
- 313 [43] Olowe OA, Olowe RA, Oluremi AS, Olusolabomi J. A novel report of colistin-resistant   
314 *Escherichia coli* carrying mcr-1 gene from animal and human feecal samples in Nigeria. Pan   
315 African J Life Sci 2018;1:7–10.
- 316 [44] KO Zubair, Iregbu K. Resistance Pattern and Detection of Metallo-beta-lactamase Genes in   
317 Clinical Isolates of *Pseudomonas aeruginosa* in a Central Nigeria Tertiary Hospital. Niger J Clin   
318 Pract 2018;21:176–82.
- 319 [45] Odewale G, Adefioye OJ, Ojo J, Adewumi FA, Olowe OA. Multidrug resistance of

320 *Acinetobacter baumannii* in Ladoke Akintola University Teaching Hospital, Osogbo, Nigeria .  
321 *Eur J Microbiol Immunol* 2016;6:238–43. <https://doi.org/10.1556/1886.2015.00018>.

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Table 1: Distribution of ESBLs producing and carbapenems resistant *Enterobacteriaceae* isolates

	Number of 3GC resistant isolates	SHV	TEM	CTX-M	Number of CRE
<i>Citrobacter freundii</i>	10	0	0	2	3
<i>Enterobacter cloacae</i>	18	0	3	5	6
<i>Escherichia coli</i>	51	2	9	26	8
<i>Klebsiella pneumoniae</i>	28	5	4	10	2
<i>Morganella morganii</i>	4	0	0	0	
<i>Proteus mirabilis</i>	14	2	1	3	
<i>Providencia rettgeri</i>	1	0	1	0	
<i>Providencia stuartii</i>	3	1	1	0	
<b>Total</b>	129	10	19	46	19

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3GC, Third generation cephalosporins; CRE, carbapenem resistant Enterobacteriaceae.

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**Table 2:** Resistance phenotypes and carbapenemase genes identified in Carbapenems resistant isolates

Strain names	Hospital	Isolation sources	<i>Bla</i> <sub>GENES</sub>	ERT MIC (µg/ml)	IPM MIC (µg/ml)	Antibiotics resistance phenotype													
<i>Escherichia coli</i> 13	SHS	Stool	<i>bla</i> <sub>OXA-181</sub>	0.75 (R)	0.38 (S)	AMX	AMC	FEP	TZP	KF	ERT	SXT	CIP	DO					
<i>Escherichia coli</i> 425	UDUTH	Urine	<i>bla</i> <sub>OXA-181</sub>	0.75 (R)	1.5 (S)	AMX	AMC	FEP	TZP	KF	ERT	SXT	CIP	DO					
<i>Citrobacter freundii</i> 448	UDUTH	Urine	<i>bla</i> <sub>NDM-5</sub>	2 (R)	6 (R)	AMX	AMC	FEP	TZP	KF	CRO	ERT	IMP	SXT	CIP	DO	CN		
<i>Citrobacter freundii</i> 167	UDUTH	Urine	<i>bla</i> <sub>NDM-5</sub>	4 (R)	6 (R)	AMX	AMC	FEP	TZP	KF	CRO	ERT	IMP	SXT	CIP	DO	CN		
<i>Enterobacter cloacae</i> 58	UDUTH	Urine	<i>bla</i> <sub>NDM-5</sub>	8 (R)	16 (R)	AMX	AMC	FEP	TZP	KF	CRO	ERT	IMP	SXT	CIP	DO	F	CN	
<i>Enterobacter cloacae</i> 116	UDUTH	Urine	<i>bla</i> <sub>NDM-5</sub>	8 (R)	12 (R)	AMX	AMC	FEP	TZP	KF	CRO	ERT	IMP	SXT	CIP	DO	AK		
<i>Enterobacter cloacae</i> 138	SHS	Sputum	<i>bla</i> <sub>NDM-5</sub>	4 (R)	8 (R)	AMX	AMC	FEP	TZP	KF	CRO	ERT	IMP	SXT	CIP	F	CN		

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329 SHS, Specialist Hospital Sokoto; UDUTH, Usmanu Danfodiyo University Teaching Hospital Sokoto. AMX, Amoxicillin; AMC, Amoxicillin-clavulanic  
 330 acid; FEP, Cefepime; CRO, Ceftriaxone; KF, Cefalotin; CN, Gentamicin; AK, Amikacin; DO, Doxycycline; CIP, Ciprofloxacin; ETP, Ertapenem; IPM,  
 331 Imipenem; SXT, Trimethoprim-Sulfamethoxazole; FF, Fosfomycin; F, Nitrofurantoin. MIC, minimum inhibitory concentration; R, resistant; S, Susceptible