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► **To cite this version:**

J. -M. Rolain, S. M. Diene, A. Olowo-Okere, Y. K. E. Ibrahim, B. O. Olayinka, et al.. Phenotypic and genotypic characterization of clinical carbapenem-resistant Enterobacteriaceae isolates from Sokoto, northwest Nigeria. *NEW MICROBES AND NEW INFECTIONS*, 2020, 37, 10.1016/j.nmni.2020.100727 . hal-03149716

HAL Id: hal-03149716

<https://amu.hal.science/hal-03149716>

Submitted on 5 Sep 2022

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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*.

1 **Abstract:**

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

20 **Keywords:** Carbapenem resistance, Carbapenemase genes, *bla_{NDM-5}*, *bla_{OXA-181}*, Nigeria

21

22 Introduction

23 Carbapenems are highly effective β -lactam antibiotics introduced into clinical practices
24 following the emergence of plasmids encoding for extended-spectrum β -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 β -lactamases [3]. The transferable plasmid encoded
28 carbapenemase (*bla*_{IMP-1}) 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 β -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*_{KPC} is the most common
41 carbapenemase genes, though recent emergence of *bla*_{NDM} producing *Enterobacteriaceae* has been
42 reported particularly among patients returning from *bla*_{NDM} endemic region [14]. Moreover,
43 despite that wide geographical variation exists in the types of carbapenemase genes in Europe,
44 generally *bla*_{KPC} and *bla*_{OXA-48} 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

47 phenotypic testing [17,18]. However, a few studies have used genotypic methods to establish the
48 occurrence of carbapenemase genes among clinical and non-clinical bacterial isolates in Nigeria
49 [19,20]. In Sokoto however, there was no data on the prevalence of CPE. In view of this, we
50 therefore aimed to investigate the prevalence of clinical *Enterobacteriaceae* isolates bearing
51 carbapenemase genes in two-leading hospitals in Sokoto, Northwest-Nigeria and also to
52 characterise their molecular resistance mechanisms.

53 **Materials and methods**

54 **Study area, design and period**

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

63 **Bacterial collection and identification**

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

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

73 **Antibiotic Susceptibility Test**

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

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

91 The phenotypic detection of ESBL enzymes was performed using the double-disk synergy
92 test by placing a β -lactamase inhibitor (amoxicillin-clavulanic and piperacillin-tazobactam) discs
93 between two third generation cephalosporins (3GCs) at a distance of 20 mm centre-to-centre.[24]

94 Formation of a characteristic keyhole effect or champagne-cork shaped zone of inhibition between
95 the discs was considered as a phenotypic indication of ESBL production. The carbapenem

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

98

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

100 The 3GC and carbapenem resistant isolates were screened for the presence of genes
101 encoding **ESBLs** (*bla*_{CTX-M}, *bla*_{TEM}, *bla*_{SHV}) and carbapenemases (*bla*_{OXA-48}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{NDM}
102 and *bla*_{KPC}), using the quantitative real-time PCR (qPCR) as previously described [26]. The qPCR
103 positive isolates were confirmed by conventional PCR. The genetic variant of the carbapenemase
104 genes was determined by sequencing of the positive PCR amplicons in both directions using the
105 same set of standard PCR primers with BigDye Terminator on an automated ABI 3500XL genetic
106 analyser (Applied Biosystems, Foster City, CA) according to the previously protocol described
107 [27]. The generated raw read sequences were assembled using codon code aligner, v 9.0.1 (Codon
108 Code Corp., Massachusetts, USA). The assembled sequences were identified by Blast analysis
109 against the ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation) database [28].

110 **Genotypic investigation of colistin resistance mechanism**

111 Moreover, one of the leading objectives of this work was to investigate colistin resistance
112 in these clinical isolates. Irrespective of the results of phenotypic colistin resistance test, qPCR
113 was used to screen the whole collection of the 292 *Enterobacteriaceae* isolates for mobilized
114 colistin resistance (*mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5* and *mcr-8*) genes, as previously described
115 [29].

116 **Result**

117 The distribution of the clinical isolates recovered during clinical diagnostic testing in the
118 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. morgani* (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_{SHV}*, *bla_{TEM}* and *bla_{CTX-M}* genes,
129 respectively (Table 1). While 9 and 8 of the isolates co-harboured *bla_{TEM}* and *bla_{CTX-M}* and *bla_{SHV}*
130 and *bla_{CTX-M}*, 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_{NDM-5}* (n=5) and *bla_{OXA-181}* (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.

144 Discussion

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

164 In this study, the presence of *bla*_{NDM-5} and *bla*_{OXA-181} accounted for carbapenem resistance
165 in about a third of the CRE isolates. This corresponds to the findings of previous studies in
166 different regions of Nigeria where *bla*_{NDM-5}, *bla*_{OXA-48} and *bla*_{OXA-181} have been reported as the
167 commonest carbapenemase genes [38,39]. Our findings however contrasted the report of a study
168 in Maiduguri, northeast-Nigeria, where *bla*_{KPC} 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.

185 **Funding information:**

186 This work was supported by the French Government under the Investments for the Future
187 program managed by the National Agency for Research, Méditerranée Infection, 10-IAHU-03.

188 **Acknowledgment:**

189 We kindly appreciate the support given to us by the staff of Usmanu Danfodiyo University
190 Teaching Hospital and Specialist Hospital Sokoto, particularly the technical support provided by
191 Mr Ibrahim Sa'adu.

192 **Transparency declaration:**

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- β -Lactamase-1 (NDM-1) Producers among Carbapenem-Resistant *Enterobacteriaceae* in Kuwait 2016;1:1–12.
280 <https://doi.org/10.1371/journal.pone.0152638>.
281
- 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 Applied 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 fecal 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	
Total	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> _{GENES}	ERT MIC (µg/ml)	IPM MIC (µg/ml)	Antibiotics resistance phenotype														
<i>Escherichia coli</i> 13	SHS	Stool	<i>bla</i> _{OXA-181}	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> _{OXA-181}	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> _{NDM-5}	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> _{NDM-5}	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> _{NDM-5}	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> _{NDM-5}	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> _{NDM-5}	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