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# Detection and molecular characterisation of extended-spectrum β-lactamase-producing enteric bacteria from pigs and chickens in Nsukka, Nigeria☆

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ABSTRACT

*Objectives:* This study screened chickens and pigs slaughtered for human consumption for the presence and characteristics of extended-spectrum β-lactamase (ESBL)- and plasmid-encoded AmpC (pAmpC) β-lactamase-producing enteric bacteria.
*Methods:* Faecal samples from 410 broiler chickens and 100 pigs were cultured on MacConkey agar supplemented with 2 μg/mL cefotaxime. Antimicrobial resistance phenotypes of the recovered isolates were determined by disk diffusion. PCR and sequencing were performed to identify the ESBL and pAmpC gene variants and other associated resistance determinants. Genetic diversity of the isolates was analysed by phylotyping and multilocus sequence typing.
*Results:* ESBL-producing *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter asburiae* and *Providencia* spp. were isolated from 17 (4.1%) and 2 (2.0%) of the samples from chickens and pigs, respectively. One pAmpC-producing *E. coli* isolate was obtained from a chicken. Resistance to tetracycline, trimethoprim/sulfamethoxazole, chloramphenicol and gentamicin was exhibited by 95%, 80%, 60% and 55% of the ESBL/pAmpC-producing strains, respectively. *tet*(A) and *aac(3)-II* were the predominant genes detected in tetracycline- and aminoglycoside-resistant strains, respectively. *bla*$_{CTX-M}$, encoding CTX-M-15 (15 isolates) or CTX-M-1 variants (3 isolates), was present in all but one ESBL-producer, either alone or in combination with *bla*$_{SHV}$ and/or *bla*$_{TEM}$. The remaining ESBL-producer, a *Providencia* spp. recovered from a chicken, harboured *bla*$_{VEB}$. The only pAmpC-positive *E. coli* strain carried *bla*$_{CMY-2}$. The 11 ESBL-producing *E. coli* strains belonged to five lineages (ST226-A, ST3625-B1, ST10-A, ST46-A and ST58-B1).
*Conclusions:* Healthy chickens and pigs act as reservoirs of ESBL/pAmpC-producing enterobacteria that can potentially be transmitted to humans through direct contact or ingestion of contaminated meat.


## 1. Introduction

Antimicrobial resistance is a growing public-health threat worldwide, and members of the Enterobacteriaceae family are among the clinically important bacteria that are rapidly developing resistance to available antibacterial agents. The most important mechanism of resistance to third-generation cephalosporins among members of the Enterobacteriaceae, particularly *Escherichia coli* and *Klebsiella pneumoniae*, is the production of extended-spectrum β-lactamase (ESBL) enzymes [1,2]. These enzymes hydrolyse the β-lactam ring of extended-spectrum β-lactam antibacterial agents (cephalosporins and monobactams). ESBL-producing bacteria pose a serious therapeutic challenge as they are frequently resistant to other classes of antimicrobial agents such as fluoroquinolones, aminoglycosides and trimethoprim/sulfamethoxazole (SXT) [3].

Three major families of ESBL enzymes have been reported, namely TEM, SHV and CTX-M types [4]. The TEM- and SHV-type ESBLs arise by point mutations leading to substitutions of key amino acid residues in the classical TEM-1/TEM-2 and SHV-1

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β-lactamases [1,2]. These ESBL types predominated in the 1980s and 1990s. However, the first non-TEM/SHV β-lactamase-producing cefotaxime-resistant *E. coli* was isolated from the faecal microbiota of a laboratory dog in Japan in 1986 [5]. The enzyme responsible for the cefotaxime resistance was designated FEC-1 and was later found to be related to CTX-M-3 type [6]. The CTX-M-type ESBLs have now emerged as the dominant types [1] and are increasingly being detected both in human and animal populations in many parts of the world [7,8]. On the basis of amino acid sequence, five groups or clusters of CTX-M-type ESBLs have been identified, namely CTX-M-1, -2, -8, -9 and -25 [2]. Healthy food animals are increasingly being reported as reservoirs of ESBL-producing enteric bacteria [7,9].

In Nigeria, most of the available reports on molecular characterisation of ESBL genes are on human isolates [10,11], with scant information on animal isolates [12,13]. The objective of this study was to screen broiler chickens and pigs slaughtered for human consumption at Ikpa market in Nsukka (Nigeria) for ESBL-producing enteric bacteria and to characterise the recovered isolates.

## 2. Materials and methods

### *2.1. Isolation and identification of extended-spectrum β-lactamase- and plasmid-encoded AmpC-producing enteric bacteria*

Faecal swab samples were collected from 410 broiler chickens (210 samples between April–June 2014 and 200 samples between September–November 2015) and 100 pigs (September–November 2015) presented for slaughter at Ikpa market/slaughterhouse. Samples were collected once a week during the sampling period, and not more than 20 chickens and 10 pigs were sampled at each visit; each sampled animal came from a different farm or owner. Swab samples were obtained from the cloaca and rectum of chickens and pigs, respectively, just before slaughter. Each swab sample was placed into a sterile tube, was transported to the laboratory and was processed within 2 h of collection. Samples were inoculated on MacConkey agar supplemented with 2 μg/mL cefotaxime (CMCA) plates and inoculated plates were incubated at 37 °C for 18–24 h. One representative colony of each morphological type from the CMCA plate was picked and was subcultured on MacConkey agar to obtain a pure culture. The pure cultures colonies were screened for ESBL production using the combination disk method [cefpodoxime/clavulanic acid (10:1 μg) and cefpodoxime alone (10 μg)] on Mueller–Hinton agar. Each test isolate producing an inhibition zone diameter difference of ≥5 mm between the combination disk and the cefpodoxime disk was considered an ESBL-producer [14]. Each isolate producing a zone difference of <5 mm was screened for susceptibility to cefoxitin (30 μg) by the disk diffusion method. Cefoxitin-resistant isolates were considered as presumptive AmpC-producers. The ESBL- and presumptive AmpC-producers were subcultured on brain–heart infusion agar, were incubated overnight at 37 °C and were processed for species identification using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) [15] and 16S rRNA sequencing. Bacterial genomic DNA was extracted using an InstaGene™ Matrix Kit (Bio-Rad, Hercules, CA) and the PCR assay was performed using previously described primers (uni16-F, AGAGTTTGATYMTGGCTCAG; and uni16-R, GGYTACCTTGTTACGACTT) [16,17]. For further molecular analysis, we only focused on bacteria producing acquired β-lactamases, i.e. ESBLs and plasmid-encoded AmpC (pAmpC) β-lactamases. Chromosomally-encoded AmpC β-lactamases were excluded.

### *2.2. Antimicrobial susceptibility testing of extended-spectrum β-lactamase- and plasmid-encoded AmpC-producing enteric bacteria*

Antimicrobial susceptibility profiles of the bacterial isolates were determined by the disk diffusion method using 13 antimicrobial agents: ampicillin (10 μg); amoxicillin/clavulanic acid (30 μg); cefoxitin (30 μg); ceftazidime (30 μg); cefotaxime (30 μg); imipenem (10 μg); nalidixic acid (30 μg); ciprofloxacin (5 μg); chloramphenicol (10 μg); SXT (25 μg); gentamicin (10 μg); tobramycin (10 μg); and tetracycline (30 μg). The results of antimicrobial susceptibility testing were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) for veterinary pathogens [18].

### *2.3. Detection of extended-spectrum β-lactamase- and plasmid AmpC-encoding genes and other antimicrobial resistance genes*

ESBL-producing bacteria were screened for the presence of genes encoding TEM-, SHV-, OXA-1- and CTX-M-type β-lactamases by PCR using specific primers and conditions reported previously [9]. Detection of acquired *ampC* genes among presumptive AmpC-producing isolates was done using a multiplex PCR assay [19]. Absence of the classic ESBL genes in an isolate prompted the search for minor ESBL families such as VEB [20]. The amplicons obtained were sequenced and the sequences were compared with those in the GenBank and Lahey Clinic (http://www.lahey.org/Studies/) public databases to identify β-lactamase variants. Tetracycline- and gentamicin-resistant isolates were screened by PCR for the presence of *tet*(A), *tet*(B), *tet*(C) and *tet*(D) genes and *aac(3)-II* genes, respectively [21].

### *2.4. Multilocus sequence typing (MLST) and phylotyping of extended-spectrum β-lactamase- and plasmid-encoded AmpC-producing Escherichia coli strains*

To identify the genetic lineages of the ESBL- and pAmpC-producing *E. coli* strains, DNA sequencing of the internal fragments of seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) was performed. The allelic profile of each strain was determined and the sequence type (ST) was assigned in accordance with the *E. coli* MSLT website (http://mlst.ucc.ie/mlst/dbs/Ecoli). The *E. coli* strains were classified into one of the seven major phylogenetic groups (A, B1, B2, C, D, E and F) using the quadruplex PCR method proposed by Clermont et al. [22].

## 3. Results and discussion

### *3.1. Extended-spectrum β-lactamase- and plasmid-encoded AmpC-producing enteric bacterial species isolated*

Samples from 18 (4.4%) of the 410 broiler chickens and 2 (2.0%) of the 100 pigs yielded growth on CMCA plates. Of the 20 isolates recovered on CMCA, 19 were phenotypically confirmed as ESBL-producers by the combination disk method using cefpodoxime/clavulanic acid and cefpodoxime disks. The remaining isolate was resistant to cefoxitin and was therefore a presumptive AmpC-producer. The isolates recovered on CMCA were identified into four species, namely *E. coli* (11 strains), *K. pneumoniae* (7 strains), *Enterobacter asburiae* (1 strain) and *Providencia* spp. (1 strain) (Table 1). Ten of the *E. coli*, all seven *K. pneumoniae* and the single *E. asburiae* and *Providencia* spp. strains were ESBL-producers, whilst one *E. coli* strain was a pAmpC-producer. Among the genera of the family Enterobacteriaceae, ESBL production is predominantly found in *E. coli* and *Klebsiella* spp. [2].

### 3.2. Resistance phenotypes of the extended-spectrum β-lactamase- and plasmid-encoded AmpC-producing Enterobacteriaceae

The resistance phenotypes of the ESBL- and pAmpC-producing strains to the antimicrobial agents tested are presented in Table 1. All of the bacterial strains studied were resistant to ceftazidime and cefotaxime; one strain was resistant to cefoxitin. Resistance to tetracycline, SXT, chloramphenicol and gentamicin was exhibited by 19 (95%), 16 (80%), 12 (60%) and 11 (55%) of the ESBL-producing strains, respectively. Moreover, 19 of the isolates were resistant to at least three classes of antimicrobial agents tested, with 8 (40%) of them were resistant to 9 or more of the 13 antimicrobial agents used. Extensive use of antimicrobial agents, particularly β-lactams and tetracyclines, in poultry production in Nigeria [23,24] may be responsible for the emergence of multidrug-resistant (MDR) enteric bacteria. Moreover, the high resistance rates recorded in this study are in accordance with data reported in food animals by other authors both from Africa and Europe [25–27]. The level of use of specific antimicrobial agents was found to be strongly correlated with the level of resistance to these agents in commensal *E. coli* isolates from cattle, pigs and poultry [26].

### 3.3. Molecular characterisation of extended-spectrum β-lactamase- and plasmid AmpC-encoding genes and other antimicrobial resistance genes

PCR screening of the 19 phenotypically confirmed ESBL-producing isolates for $bla_{CTX}$, $bla_{TEM}$ and $bla_{SHV}$ genes showed that $bla_{CTX-M}$ was present in all but one ESBL-producer, either alone (10 isolates) or in combination with $bla_{SHV}$ (4 isolates), $bla_{TEM}$ (2 isolates) or $bla_{SHV}$ + $bla_{TEM}$ (2 isolates) (Table 1). The remaining ESBL-producer, a *Providencia* spp. isolate, harboured a minor ESBL gene ($bla_{VEB}$). Thus, ESBL-producing enteric bacteria were isolated from 17 (4.1%) of the 410 chicken samples and 2 (2.0%) and the 100 pig samples. The higher rate of ESBL-producing isolates among broiler chickens is in accordance with data reported from Tunisia [27]. In Ibadan (Nigeria), ESBL-producing *E. coli* was detected in one of the faecal samples collected from 100 chickens at slaughter, whilst none of the 100 samples from pigs were positive [28]. The variability in the rate of detection of ESBL-producing enteric bacteria may be explained in part by differences in the methodology used. This can also be partially explained by the fact that antibiotic usage is growing in intensive farming, more common in poultry where animals are reared in close proximity [7].

**Table 1**
Phenotypic and molecular characteristics of extended-spectrum β-lactamase (ESBL)- and plasmid AmpC (pAmpC) β-lactamase-producing Enterobacteriaceae isolated from chickens and pigs in Nsukka, Nigeria.

| Strain no. | Identity | Source | Resistance phenotype | β-Lactamase gene (s) | Other antimicrobial resistance genes | Phylogroup (*E. coli*) | MLST (*E. coli*) |
|---|---|---|---|---|---|---|---|
| C6086 | *Escherichia coli* | Broiler chicken | AMP, CTX, CAZ, TET, SXT | CTX-M-15 | *tet*(A) | A | ST226 |
| C6089 | *E. coli* | Broiler chicken | AMP, CTX, CAZ, TET, SXT, NAL | CTX-M-15 | *tet*(A) | A | ST226 |
| C8830 | *E. coli* | Broiler chicken | AMP, CTX, CAZ, TET, SXT | CTX-M-15 | | A | ST226 |
| C8833 | *E. coli* | Broiler chicken | AMP, CTX, CAZ, TET, SXT | CTX-M-15, TEM-1 | | A | ST226 |
| C6087 | *E. coli* | Broiler chicken | AMP, AMC, CTX, CAZ, CHL, TET, SXT, NAL, CIP, GEN, TOB | CTX-M-15 | *tet*(B), *aac(3)-II* | A | ST10 |
| C6088 | *E. coli* | Broiler chicken | AMP, AMC, CTX, CAZ, CHL, TET, SXT, NAL, CIP, GEN, TOB | CTX-M-15 | *tet*(B), *aac(3)-II* | A | ST10 |
| C8835 | *E. coli* | Pig | AMP, AMC, CTX, CAZ, CHL, TET, SXT | CTX-M-15 | | A | ST46 |
| C6090 | *E. coli* | Broiler chicken | AMP, CTX, CAZ, TET, NAL | CTX-M-1 | *tet*(A) | B1 | ST3625 (new) |
| C6091 | *E. coli* | Broiler chicken | AMP, CTX, CAZ, TET, NAL | CTX-M-1 | *tet*(A) | B1 | ST3625 (new) |
| C6092 | *E. coli* | Broiler chicken | AMP, CTX, CAZ, TET, NAL | CTX-M-1 | *tet*(A) | B1 | ST3625 (new) |
| C8831 | *E. coli* | Broiler chicken | AMP, AMC, CTX, CAZ, FOX | CMY-2 | | B1 | ST58 |
| C6093 | *Enterobacter asburiae* | Broiler chicken | AMP, AMC, CTX, CAZ, CHL, TET, SXT, NAL, GEN, TOB | CTX-M-15 | *tet*(A) | NT | NT |
| C6094 | *Klebsiella pneumoniae* | Broiler chicken | AMP, AMC, CTX, CAZ, CHL, TET, SXT, NAL, CIP, GEN, TOB | CTX-M-15, SHV | *aac(3)-II* | NT | NT |
| C6095 | *K. pneumoniae* | Broiler chicken | AMP, AMC, CTX, CAZ, CHL, TET, SXT, NAL, CIP, GEN, TOB | CTX-M-15, SHV | *aac(3)-II* | NT | NT |
| C8829 | *K. pneumoniae* | Pig | AMP, AMC, CTX, CAZ, CHL, TET, SXT, GEN, TOB | CTX-M-15, SHV | *aac(3)-II* | NT | NT |
| C8834 | *K. pneumoniae* | Broiler chicken | AMP, CTX, CAZ, CHL, TET, SXT, NAL, CIP, GEN, TOB | CTX-M-15, SHV, TEM-1 | *aac(3)-II* | NT | NT |
| C8836 | *K. pneumoniae* | Broiler chicken | AMP, CTX, CAZ, CHL, TET, SXT, GEN, TOB | CTX-M-15, SHV | *aac(3)-II* | NT | NT |
| C8837 | *K. pneumoniae* | Broiler chicken | AMP, CTX, CAZ, CHL, TET, SXT, GEN, TOB | CTX-M-15, TEM-1 | *tet*(A), *aac(3)-II* | NT | NT |
| C8838 | *K. pneumoniae* | Broiler chicken | AMP, CTX, CAZ, CHL, TET, SXT, NAL, CIP, GEN, TOB | CTX-M-15, SHV, TEM-1 | *aac(3)-II* | NT | NT |
| C8832 | *Providencia* spp. | Broiler chicken | AMP, CTX, CAZ, CHL, TET, SXT, GEN, TOB | VEB | *aac(3)-II* | NT | NT |

MLST, multilocus sequence typing; AMP, ampicillin; CTX, cefotaxime; CAZ, ceftazidime; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; NAL, nalidixic acid; AMC, amoxicillin/clavulanic acid; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; FOX, cefoxitin; NT, not tested.

The $bla_{SHV}$ gene was detected in 6 of the 7 *K. pneumoniae* isolates studied, whilst $bla_{TEM-1}$ was found in 3 of the 7 *K. pneumoniae* and 1 of the 11 *E. coli* isolates. Detection of the $bla_{SHV}$ gene among *K. pneumoniae* is not surprising and is likely in relation with the production of SHV-1 β-lactamase usually chromosomally encoded in this species [29].

DNA sequencing of the $bla_{CTX}$ genes revealed two CTX-M types (CTX-M-15 and CTX-M-1), with CTX-M-15 being the predominant CTX-M-type ESBL, detected in 15 of the 19 ESBL-positive isolates in this study. Thus, irrespective of the source of the sample, CTX-M-15 was the predominant CTX-M type reported among members of the Enterobacteriaceae in Nigeria. CTX-M-15 and CTX-M-1 (both belonging to CTX-M-1 cluster) detected in this study are among the most widespread and predominant CTX-M types reported in humans and various animal species in many regions of the world, including the African continent [7,30]. VEB β-lactamase is a rare type of enzyme responsible for conferring high-level resistance to expanded-spectrum cephalosporins. The $bla_{VEB}$ gene has been identified in a variety of species of Enterobacteriaceae and non-fermenting Gram-negative bacilli. The first description of a $bla_{VEB}$ gene from Africa was reported in Algeria, in a MDR clinical isolate of *Providencia stuartii* [31]. Later, another *P. stuartii* strain producing VEB β-lactamase was reported from a hospitalised patient in Tunisia [32]. However, to our knowledge, this is the first report on the carriage of the $bla_{VEB}$ gene in a bacteria of animal origin in Africa.

The only cefoxitin-resistant *E. coli* strain (isolated from a chicken) carried $bla_{CMY-2}$ that encodes the production of plasmidic class C or pAmpC β-lactamase enzyme. The $bla_{CMY-2}$ gene was first described in *K. pneumoniae* in Greece and is less frequently detected among third-generation cephalosporin-resistant Enterobacteriaceae than ESBL-encoding genes [33]. However, different studies from northern Africa reported considerably high rates of pAmpC β-lactamase production (all corresponding to the CMY-2 variant) among commensal *E. coli* recovered from healthy and septicaemic broilers [27,34]. Interestingly, CMY-2 and DHA-1 pAmpC variants are considered the most prevalent among human clinical isolates in Africa [35]. *tet*(A) and *aac(3)-II* were the predominant genes detected in the tetracycline- resistant and aminoglycoside-resistant ESBL strains, respectively.

### 3.4. Multilocus sequence typing and phylogrouping of extended-spectrum β-lactamase- and plasmid-encoded AmpC-producing *Escherichia coli* strains

The 11 ESBL- and pAmpC-producing *E. coli* strains analysed belonged to five sequence types, namely ST226 (4 strains), ST3625 (3 strains), ST10 (2 strains), ST46 (1 strain) and ST58 (1 strain), with ST3625 being reported for the first time in this study. The three strains with this new sequence type were isolated from broiler chickens and they all had a similar resistance phenotype, harboured the $bla_{CTX-M-1}$ gene and belonged to phylogroup B1. Apart from the CMY-2-producing *E. coli* strain that belonged to phylogroup B1 as well as the three ST3625 strains mentioned above, all of the other strains studied belonged to phylogroup A (Table 1). Thus, the ESBL-producing *E. coli* strains studied predominantly belonged to phylogroup A (7/11). All seven phylogroup A strains were CTX-M-15-type ESBL-producers and were distributed among three sequence types. The *E. coli* strains in the current study essentially belonged to the phylogroups associated with commensal strains [22] and, as previously pointed out [36], this may be due to the fact that all were recovered from faecal samples. The results of phylogenetic grouping and MLST indicated that the ESBL-producing *E. coli* from chickens and pigs in the study area belonged to diverse genetic lineages. Although many clinical *E. coli* strains expressing ESBLs of the CTX-M group have been associated with phylogroups B2 and D, mainly in relation to expansion of the pandemic *E. coli* ST131-B2 and other clones such as ST405-D and ST38-D, the strains from livestock origin involved in the present study belonged to phylogroups A and B1. However, the *E. coli* ST10-A clone detected in two broiler chickens has also been reported among commensal and pathogenic *E. coli* from humans [37,38], domesticated animals [7,39] and birds [40]. This clone appears to be associated with the dissemination of different CTX-M enzyme groups (CTX-M-1, CTX-M-2 and CTX-M-9) in various settings [39]. Furthermore, the CMY-2-producing *E. coli* isolate was assigned to ST58-B1, a clone widely spread both in humans and animals in relation to the production of CMY-2 and CTX-M-1 group enzymes [34,39,41]. The results of this study, as well as those of previous studies, show the association between specific clones and ESBL/pAmpC genetic variants and highlighted their apparent continuous flow between human and animal settings.

## 4. Conclusion

MDR ESBL- and pAmpC-producing members of the Enterobacteriaceae family are present in chickens and pigs slaughtered for human consumption in Nsukka, Nigeria. The predominant ESBL gene type in this study was $bla_{CTX-M-15}$, and three of the *E. coli* isolates belonged to a new sequence type (ST3625). The pAmpC β-lactamase CMY-2 and the minor ESBL type VEB are reported here for the first time from food animals in Nigeria and Africa, respectively. Healthy chickens and pigs may act as reservoirs of ESBL/pAmpC-producing enterobacteria isolates that can be potentially transmitted to humans through direct contact or ingestion of derived contaminated meat. Association of members of the Enterobacteriaceae family from food-producing animals with genes encoding antimicrobial resistance constitutes a serious public-health concern.

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## Competing interests

None declared.

## Ethical approval

Not required.

## References

[1] Paterson D.L., Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev 2005;18:657–86.
[2] Pitout JD, Laupland KB. Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public health concern. Lancet Infect Dis 2008;8:159–66.
[3] Coque TM, Baquero F, Cantón R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill 2008;13:19–44.
[4] Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrob Agents Chemother 2010;54:969–76.
[5] Matsumoto Y, Ikeda F, Kamimura T, Yolota Y, Mine Y. Novel plasmid-mediated β-lactamase from *Escherichia coli* that inactivates oxyimino-cephalosporins. Antimicrob Agents Chemother 1988;32:1243–6.
[6] Bonnet R. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 2004;48:1–14.
[7] Alonso CA, Zarazaga M, Ben Sallem R, Jouini A, Ben Slama K, Torres C. Antibiotic resistance in *Escherichia coli* in husbandry animals: the African perspective. Lett Appl Microbiol 2017;64:318–34.

[8] Cantón R, González-Alba JM, Galán JC. CTX-M enzymes: origin and diffusion. Front Microbiol 2012;3:110, doi:http://dx.doi.org/10.3389/fmicb.2012.00110.

[9] Costa D, Vinue L, Poeta P, Coelho AC, Matos M, Saenz Y, et al. Prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* isolates in faecal samples of broilers. Vet Microbiol 2009;138:339–44.

[10] Raji MA, Jamal W, Ojemeh O, Rotimi V. Sequence analysis of genes mediating extended-spectrum β-lactamase (ESBL) production in isolates of Enterobacteriaceae in a Lagos Teaching Hospital, Nigeria. BMC Infect Dis 2015;15:259, doi:http://dx.doi.org/10.1186/s12879-015-1005-x.

[11] Inwezerua C, Mendonca N, Calhau V, Domingues S, Adelike OE, Da Silva GJ. Occurrence of extended-spectrum β-lactamases in human and bovine isolates of *Escherichia coli* from Oyo State, Nigeria. J Infect Dev Ctries 2014;8:774–9.

[12] Afiukwa NF, Iroha RI, Afiukwa AC, Igwe OD. First report of $bla_{CTX\text{-}M\text{-}15}$ extended spectrum β-lactamase (ESBL) producing *E. coli* isolated from cloacal swabs of birds in South Eastern Nigeria. Int Arch Biomed Clin Res 2016;2:35–9.

[13] Olowe OA, Adewumi O, Odewale G, Ojurongbe O, Adefioye OJ. Phenotypic and molecular characterization of extended-spectrum β-lactamase producing *Escherichia coli* obtained from animal fecal samples in Ado Ekiti, Nigeria. J Environ Public Health 2015;2015:497980, doi:http://dx.doi.org/10.1155/2015/497980.

[14] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement M100, 27th ed. Wayne, PA: CLSI; 2017.

[15] Bizzini A, Jaton K, Romo D, Bille J, Prod'hom G, Greub G. Matrix assisted laser desorption ionization-time of flight mass spectrometry as an alternative to 16S rRNA gene sequencing for identification of difficult-to-identify bacterial strains. J Clin Microbiol 2011;49:693–6.

[16] Juretschko S, Timmermann G, Schmid M, Schleifer K, Pommerening-Roser A, Koop H, et al. Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: *Nitrosococcus mobilis* and *Nitrospira*-like bacteria as dominant populations. Appl Environ Microbiol 1998;64:3042–51.

[17] Loy A, Lehner A, Lee N, Adamczyk J, Meier H, Ernst J, et al. Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment. Appl Environ Microbiol 2002;68:5064–81.

[18] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard—fourth edition. CLSI document VET01-A4. Wayne, PA: CLSI; 2013.

[19] Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 2002;40:2153–62.

[20] Naas T, Benaoudia F, Massuard S, Nordmann P. Integron-located VEB-1 extended-spectrum β-lactamase gene in a *Proteus mirabilis* clinical isolate from Vietnam. J Antimicrob Chemother 2000;46:703–11.

[21] Sáenz Y, Briñas L, Domínguez E, Ruiz J, Zarazaga M, Vila J, et al. Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. Antimicrob Agents Chemother 2004;48:3996–4001.

[22] Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep 2013;5:58–65.

[23] Chah KF, Nweze NE. Antibiotic use in poultry production in Nsukka, Southeast Nigeria. Proceedings of the 26th Annual Conference of the Nigerian Society for Animal Production. Nigerian Society for Animal Production; 2001. p. 69–72.

[24] Adesokan HK, Akanbi IO, Akanbi IM, Obaweda RA. Pattern of antimicrobial usage in livestock animals in south-western Nigeria: the need for alternative plans. Onderstepoort J Vet Res 2015;82:816–21.

[25] Adenipekun EO, Jackson CR, Oluwadun A, Iwalokun BA, Frye JG, Barrett JB, et al. Prevalence and antimicrobial resistance in *Escherichia coli* from food animals in Lagos, Nigeria. Microb Drug Resist 2015;21:358–65.

[26] Chantziaras I, Boyen F, Callens B, Dewulf J. Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: a report on seven countries. J Antimicrob Chemother 2014;69:827–34.

[27] Ben Sallem R, Ben Slama K, Saenz Y, Rojo-Bezares B, Estepa V, Jouini A, et al. Prevalence and characterization of extended spectrum β-lactamase (ESBL)- and CMY-2-producing *Escherichia coli* isolates from healthy food-producing animals in Tunisia. Foodborne Pathogen Dis 2012;9:1137–42.

[28] Fortini D, Fashae K, García-Fernández A, Villa L, Carattol A. Plasmid-mediated quinolone resistance and β-lactamases in *Escherichia coli* from healthy animals from Nigeria. J Antimicrob Chemother 2011;66:1269–72.

[29] Chaves J, Ladona MG, Segura C, Coira A, Reig R, Ampurdanés C. SHV-1 β-lactamase is mainly a chromosomally encoded species-specific enzyme in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2001;45:2856–61.

[30] Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH. Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 2012;18:646–55.

[31] Aubert D, Naas T, Lartigue MF, Nordmann P. Novel genetic structure associated with an extended-spectrum β-lactamase $bla_{VEB}$ gene in a *Providencia stuartii* clinical isolate from Algeria. Antimicrob Agents Chemother 2005;49:3590–2.

[32] Mahrouki S, Chihi H, Bourouis A, Ben Moussa M, Belhadj O. First characterization of a *Providencia stuartii* clinical isolate from a Tunisian intensive care unit coproducing VEB-1-a, OXA-2, *qnrA6* and *aac(6′)-Ib*-cr determinants. Braz J Infect Dis 2014;18:211–4.

[33] Philippon A, Arlet G, Jacoby GA. Plasmid-determined AmpC-type β-lactamases. Antimicrob Agents Chemother 2002;46:1–11.

[34] Maamar E, Hammami S, Alonso CA, Dakhli N, Abbassi MS, Ferjani S, et al. High prevalence of extended-spectrum and plasmidic AmpC β-lactamase-producing *Escherichia coli* from poultry in Tunisia. Int J Food Microbiol 2016;231:69–75.

[35] Storberg V. ESBL-producing Enterobacteriaceae in Africa—a non-systematic literature review of research published 2008–2012. Infect Ecol Epidemiol 2014;4:50–5.

[36] Cristovao F, Alonso CA, Igrejas G, Sousa M, Silva V, Pereira JE, et al. Clonal diversity of extended-spectrum β-lactamase producing *Escherichia coli* isolates in fecal samples of wild animals. FEMS Microbiol Lett 2017;364:, doi:http://dx.doi.org/10.1093/femsle/fnx039.

[37] Peirano G, van der Bij AK, Gregson DB, Pitout JD. Molecular epidemiology over an 11-yearperiod (2000 to 2010) of extended-spectrum β-lactamase-producing *Escherichia coli* causing bacteremia in a centralized Canadian region. J Clin Microbiol 2012;50:294–9.

[38] Okeke IN, Wallace-Gadsden F, Simons HR, Matthews N, Labar AS, Hwang J, et al. Multi-locus sequence typing of enteroaggregative *Escherichia coli* isolates from Nigerian children uncovers multiple lineages. PLoS One 2010;5:e14093.

[39] Day MJ, Rodríguez I, van Essen-Zandbergen A, Dierikx C, Kadlec K, Schink AK, et al. Diversity of STs, plasmids and ESBL genes among *Escherichia coli* from humans, animals and food in Germany, the Netherlands and the UK. J Antimicrob Chemother 2016;71:1178–82.

[40] Báez J, Hernández-García M, Guamparito C, Díaz S, Olave A, Guerrero K, et al. Molecular characterization and genetic diversity of ESBL-producing *Escherichia coli* colonizing the migratory Franklin's gulls (*Leucophaeus pipixcan*) in Antofagasta, North of Chile. Microb Drug Resist 2015;21:111–6.

[41] Cortés-Cortés G, Lozano-Zarain P, Torres C, Castañeda M, Sánchez GM, Alonso CA, et al. Detection and molecular characterization of *Escherichia coli* strains producers of extended-spectrum and CMY-2 type β-lactamases, isolated from turtles in Mexico. Vector Borne Zoonotic Dis 2016;16:595–603.