Antimicrobial use and detection of cefotaxime-resistant Enterobacteriaceae in the pig production chain, Ogun State, Nigeria

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Keywords

Swine, *Escherichia coli, Klebsiella pneumoniae*, Enterobacteriaceae, antimicrobial resistance, cefotaxime, Nigeria

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Summary

Cefotaxime belongs to the third-generation cephalosporin group of antimicrobials, which are classified as critical for the treatment of infections in humans. The upsurge in the incidence of cefotaxime-resistant (C-R) bacteria from animal sources is of global public health importance. This study investigated the presence of C-R Enterobacteriaceae in the pig production chain in Ogun State, Nigeria, and examined C-R isolates for production of extended-spectrum β-lactamase (ESBL). The knowledge, attitude and practices of pig farmers regarding antimicrobial usage were also investigated. C-R bacteria were detected in 54 (17.8%) out of 303 samples. C-R isolates were identified as *Escherichia coli* (n = 22), *Klebsiella* spp. (n = 17), *Enterobacter aerogenes* (n = 10) and *Citrobacter freundii* (n = 5). The organisms were present in feces from on-farm pigs (15/109; 13.7%), fresh pork at slaughterhouses (19/40; 47.5%), frozen pork at retail shops (7/28; 25.0%), cutting surfaces of butchers' tools (7/52; 13.5%), and abattoir effluent water (6/41; 14.6%). No C-R bacteria were detected in ready-to-eat pork. Three isolates of *Es. coli* and one of *K. pneumoniae* were ESBL-producers and possessed $bla_{CTX\text{-}M\text{-}15}$ ESBL gene variant. ESBL-producing *Es. coli* belonged to phylogenetic group A. All C-R isolates were resistant to more than three antimicrobials from different classes of antimicrobials. Tetracycline, ampicillin, amoxicillin, ciprofloxacin and enrofloxacin were among the commonly used antimicrobials in pig production, whereas cephalosporins were rarely used. Farmers knew that pigs could serve as reservoirs of pathogenic bacteria transmissible to humans. However, they were not aware that the use of antimicrobials in pig production could lead to the development and proliferation of antimicrobial-resistant bacteria in pigs. Efforts should be made to improve awareness among farmers on the roles of antimicrobial use in the emergence and dissemination of antimicrobial-resistant bacteria in animal production.

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■ INTRODUCTION

Cefotaxime is a broad-spectrum third-generation cephalosporin (Klein and Cunha, 1995). It has been used extensively for the treatment of infections caused by bacteria that are refractory to treatment with the older generation cephalosporins and penicillins (Neu, 1982). Subsequent to the first report of transferrable resistance to cefotaxime and other third and fourth generation cephalosporins among clinical bacterial isolates (Knothe et al., 1983; Sirot et al., 1987), there have been increasing reports of global emergence and widespread dissemination of resistant bacterial strains that harbor extended-spectrum cefotaximase from human, animal and environmental sources (Rossolini et al., 2008; Bevan et al., 2017).

Extensive use of cephalosporins might have contributed to the selection and proliferation of plasmids encoding extended-spectrum beta-lactamases (ESBLs) (Medeiros, 1997). It is also worrisome that such plasmids may additionally harbor genes encoding resistance to other classes of antimicrobials thereby conferring multidrug resistance traits on cefotaxime-resistant (C-R) bacteria (Canton and Coque, 2006). It is important to note that cefotaxime remains a preferred choice for the

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treatment of many complicated and uncomplicated infections such as urinary-tract infection and meningitis in humans. Indeed, the World Health Organisation listed cefotaxime among the critically important drugs (WHO, 2017). As such, the preservation of the continued efficacy of this drug is highly desirable. There are very limited options for the elimination of C-R bacteria in clinical infections.

Pig production and pork consumption contribute to the economic and nutritional well-being of many people in Nigeria (Obayelu et al., 2017). Nigeria is not self-sufficient in the supply of animal protein and pig production has enormous potential to fill the gaps. Pig has been described as the most important domestic animal in Nigeria in terms of the number of farmers engaged in its production, economic values, potentials for the supply of animal protein and role in the transmission of zoonotic infections (Ugbomoiko et al., 2008; Obayelu et al., 2017). Antimicrobials including cephalosporins are routinely used as a major input to boost animal production in Nigeria (Ojo et al., 2016a). The extensive use of third- and fourth-generation cephalosporins (3GC and 4GC) especially ceftiofur and cefquinone in food animals might have greatly facilitated the rapid development of 3GC-resistant bacteria in food animals and the spread of such resistant strains along the production and marketing chain (Cavaco et al., 2008; Wittum, 2012). There is evidence that the use of ceftiofur, cefquinone and related antimicrobials exerts selective pressure that promotes the development, proliferation and transmission of 3GC-resistant bacteria among farm animal populations and in farm environment (Cavaco et al.; 2008, Wittum, 2012). Thus, farm animals may serve as reservoirs of 3GC-resistant bacteria transmissible to humans through direct and indirect contact, contaminated environments and foods of animal origin.

Production of ESBLs is one of the major mechanisms of bacterial resistance to β-lactam antimicrobials. It confers resistance to a broad range of β-lactam antimicrobials including penicillin, cephalosporins (including 3GC) and monobactam (aztreonam) (Bradford, 2001). ESBL production can be acquired by plasmid transfer which is very common among Enterobacteriaceae. ESBL-producing bacteria are often multidrug resistant because of the colocation of resistance genes that confer resistance to other classes of antimicrobials on the ESBL-carrying plasmid. Thus, ESBL-producing bacteria play a significant role in the widespread dissemination of antimicrobial resistance among farm animals (Okpara et al., 2018). The use of third- or fourth-generation cephalosporin has been associated with increased detection of ESBL-producing *Escherichia coli* in pigs (Hammerum et al., 2014).

The present study investigated the occurrence of C-R Enterobacteriaceae in sources along the pig production chain from farms, slaughterhouses and markets in Ogun State, Nigeria. Cefotaxime-resistance bacteria were further examined for the production of ESBLs. Data were also collected on the knowledge, attitude and practices of pig farmers regarding antimicrobial usage in pig production.

■ MATERIALS AND METHODS

Sample collection

Samples were collected along the pig-production, pork-processing and marketing chain (hereafter called the pig-production chain) for the detection of C-R Enterobacteriaceae in six locations (Ifo, Sagamu, Oke-Aro, Abeokuta, Atan and Ijebu-Ode) in Ogun State. Six types of samples were collected (Table I): feces of on-farm pigs, fresh pork at slaughterhouses, swabs of cutting surfaces (knives and tables) at slaughterhouses, abattoir effluent water at points of discharge into natural flowing water bodies (streams and rivers), frozen pork at meat retail shops, and ready-to-eat pork from hawkers in open markets. One hundred and nine pooled fecal samples from pigs were collected irrespective of age, sex and breed in 60 farms. Two pooled fecal samples were collected from each of 49 farms whereas one pooled fecal sample was collected from each of 11 farms. Each pooled sample represented rectal swabs of 10 individual pigs from the same farm. Ten pigs were randomly sampled from every 50 pigs on each farm visited. Forty fresh and 28 frozen pork samples (5 g each) were collected from meat vendors at slaughterhouses and retail shops, respectively. Only one sample was collected from an individual vendor. Swabs of cutting surfaces were collected from 52 individual butchers at slaughterhouses. A single sterile swab was used for the knife and table of each butcher. Forty-one effluent water samples (5 ml each) from slaughterhouses (exclusively for pigs) were collected at the various points of discharge into natural flowing water bodies. Thirty-three ready-to-eat fried pork samples (5 g each) were collected from meat hawkers in open markets. Only one sample was collected from each hawker. Samples were collected aseptically (avoiding cross contamination), labeled appropriately and transported in icepacks to the laboratory for immediate microbiological analysis.

Isolation and identification of cefotaxime-resistant Enterobacteriaceae

Each sample was inoculated into buffer peptone water (BPW, Oxoid, Basingstoke, UK) and incubated at 37°C overnight for pre-enrichment. A loopful of the pre-enrichment broth culture was streaked onto MacConkey agar supplemented with ampicillin at 100 g/L ($MAC_{AMP-100}$) for the isolation of ampicillin-resistant bacteria. This was incubated at 37°C for 24 hours. Five colonies from each $MAC_{AMP-100}$ plate were inoculated onto separate MacConkey agar supplemented with cefotaxime at 1 g/L (MAC_{CTX-1}) and incubated at 37°C for 24 hours for the isolation of C-R strains. One distinct colony of C-R isolate was selected from each MAC_{CTX-1} plate. Selected isolates were preserved on nutrient agar slants for further analysis. Each selected C-R isolate was subjected to biochemical characterization for identification of Enterobacteriaceae using a commercially available biochemical kit (Oxoid Microbact GNB 24E) and interpreted using available computer software.

Phenotypic detection of ESBL-producing Enterobacteriaceae

All C-R isolates were tested for production of ESBL using the combination disk kit (Oxoid, Basingstoke) containing cefpodoxime (CPD, 10 µg) and cefpodoxime-clavulanic acid (CD 01, 10/1 µg). A fresh culture of the test organism on nutrient agar was emulsified in normal saline and adjusted to an optical density corresponding to 0.5 McFarland standard. This was spread evenly on Mueller Hinton agar (MHA) and the disks introduced firmly on the agar. The inoculated MHA was incubated at 35 ± 2°C for 16 hours. The difference in the zones of inhibition around the two disks was determined. Isolates that produced

Table I

Distribution of samples collected for the detection of cefotaxime-resistance Enterobacteriaceae in the pig production chain, Ogun State, Nigeria

Location	Feces	Fresh pork	Frozen pork	Effluent water	Ready-to-eat pork	Swab of cutting surface	Total
Ifo	12	7	4	2	5	3	32
Sagamu	17	7	6	3	5	8	43
Okearo	53	6	3	3	5	4	75
Abeokuta	22	5	5	4	7	2	40
Atan	5	5	4	11	6	8	35
Ijebu-ode	0	10	6	18	5	27	78
Total	109	40	28	41	33	52	303

differences equal to or greater than five millimeters in the diameter of zone of inhibition between cefpodoxime and cefpodoxime/clavulanic acid disks were identified as phenotypic ESBL producers (CLSI, 2018).

ESBL-gene detection and determination of phylogenetic groups of ESBL-producing Es. coli

The presence of ESBL-associated genes bla_{CTX}, bla_{SHV} and bla_{TEM} was investigated in phenotypic ESBL-producing isolates using polymerase chain reaction (PCR) assays, nucleotide sequencing and sequence analysis. Genomic deoxyribonucleic acid (DNA) was extracted from overnight tryptic soy broth culture of phenotypic ESBL-producing isolates by thermolysis according to Ojo et al. (2016b). DNA was quantified by nanospectrophotometry and adjusted to a final working concentration of 100 ng/µl. A multiplex PCR assay was used for the amplification and detection of ESBL-associated genes: $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}9}$, bla_{TEM} and bla_{SHV}. The PCR reaction mix and amplification conditions were as previously described (Gröbner et al., 2009; Cullik et al., 2010). The amplicons were electrophoresed in agarose gels stained with midori-green direct and visualized under ultraviolet transilluminator. Positive samples with targeted genes were further subjected to another round of PCR assays for sequencing of the whole $bla_{CTX\text{-}M\text{-}group1}$ and/or $bla_{CTX\text{-}M\text{-}group9}$ genes (LGC Genomic, Berlin, Germany). The nucleotide sequences were analyzed with bioinformatics software Geneious 10.1.3 (Biomatters, New Zealand). The ESBL gene variants were determined from analyzed sequences by comparing the nucleotide sequences with reference sequences from Lahey Clinic (www.lahey.org) and those deposited at the National Center for Biotechnology Information website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Furthermore, the phylogenetic groups of ESBL-producing *Es. coli* isolates were determined by a previously described PCR-based method (Clermont et al., 2013) for assigning *Es. coli* to one of seven *Es. coli sensu stricto* phylogenetic groups (A, B1, B2, C, D, E, and F).

Antimicrobial susceptibility testing

C-R isolates were tested for susceptibility to selected antimicrobials with the Kirby Bauer disk diffusion method according to the guideline of the Clinical and Laboratory Standards Institute (CLSI, 2018). The following antimicrobials were included: amikacin (AMK, 30 µg), ampicillin (AMP, 10 µg), amoxicillin/clavulanic acid (AMX, 30 µg), cefotaxime (CTX, 30 µg), cefoxitin (FOX, 30 µg), ceftazidime (CAZ, 30 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), nalidixic acid (NAL, 30 µg), gentamicin (GEN, 30 µg), kanamycin (KAN, 30 µg), streptomycin (STR, 10 µg), sulfamethoxazole (SXT, 25 µg), trimethoprim (TMP, 5 µg) and tetracycline (TET, 30 µg). A suspension of test organisms was prepared in normal saline and adjusted to a turbidity level of 0.5 McFarland standard (approximately 1×10^6 colony forming units/ml). The suspension was spread evenly on Mueller Hinton agar using a sterile cotton swab. The antimicrobial disks were placed firmly on the inoculated agar and incubated at 35 ± 2°C for 16 hours. The diameter of the zones of inhibition around each disk was measured and interpreted according to CLSI (2018). *Es. coli* ATCC 25922 was also tested for quality control.

Assessment of antimicrobial usage on pig farms

A structured questionnaire and in-depth interviews were used to collect data on knowledge, attitude and practices regarding antimicrobial usage on pig farms. Sixty farm managers/farmers or their representatives from 60 pig farms where fecal samples were collected were interviewed. Information was obtained on farm characteristics, commonly used antimicrobials, factors influencing the use of antimicrobials as well as practices regarding their administration. Farm characteristics related to farm size, management system, form of operation (breeding or fattening), year of establishment and presence of other animal species. Farmers were also asked to provide a complete list of antimicrobials routinely used on the farm. When an unfamiliar trade name of an antimicrobial was cited, the farmer was requested to provide its package in order to get the generic name of the antimicrobial(s). Questions were also asked on disease occurrence, diagnosis, management practices, and the reasons (prophylaxis, therapeutic and growth promotion) for antimicrobial use. Information was obtained on routes and duration of drug administration as well as the involvement of farm attendants in drug administration. A typical interview section lasted for about one hour.

Statistical analysis

Data were presented in absolute values and percentages. Data from different sample categories were compared with the Chi square test (significant values at $p < 0.05$).

■ RESULTS

Cefotaxime-resistant Enterobacteriaceae from the pig-production chain

Fifty-four (17.8%) of 303 samples yielded C-R isolates as follows: 15 (13.7%) from fecal samples, 19 (47.5%) from fresh pork, 7 (25.0%) from frozen pork, 6 (14.6%) from water discharge, and 7 (13.5%) from cutting surfaces (Table II). No C-R bacteria were detected in ready-to-eat pork. The rates of detection of C-R bacteria were significantly higher in fresh and frozen pork than in other sample types ($p < 0.05$). C-R isolates were identified as *Escherichia coli* (n = 22), *Klebsiella pneumoniae* (n = 17), *Enterobacter aerogenes* (n = 10), *K. oxytoca* (n = 2) and *Citrobacter freundii* (n = 5) (Table II). C-R isolates were

Table II

Cefotaxime-resistant Enterobacteriaceae isolated from sample sources along the pig-production chain, Ogun State, Nigeria

Sample source (n)	Number (%) of cefotaxime-resistant Entrobacteriaceae				
	Escherichia coli	*Klebsiella* spp.	*Enterobacter aerogenes*	*Citrobacter freundii*	All isolates
Feces (109)	7 (6.4)	4 (3.7)	2 (1.8)	2 (1.8)	15 (13.7)
Fresh pork (40)	8 (20.0)	6 (15.0)	3 (7.5)	2 (5.0)	19 (47.5)
Frozen pork (28)	3 (10.7)	1 (3.6)	2 (7.1)	1 (3.6)	7 (25.0)
Effluent water (41)	1 (2.4)	3 (7.3)	2 (4.9)	–	6 (14.6)
Ready-to-eat pork (33)	–	–	–	–	–
Swab of cutting surface (52)	3 (5.8)	3 (5.8)	1 (1.9)	–	7 (13.5)
Total (303)	22 (7.3)	17 (5.6)	10 (3.3)	5 (1.7)	54 (17.8)

detected in fecal samples from six (10.0%) out of 60 farms. The six positive farms were distributed across four out of six towns included in this study. The fresh pork samples positive for C-R isolates were obtained from 19 (47.5%) out of 40 meat vendors. The vendors with positive samples were from five out of six slaughterhouses investigated. Similarly, seven (25.0%) out of 28 frozen meat retail shops yielded positive isolates. The positive meat retail shops were from four towns. Effluent water from two slaughterhouses yielded C-R isolates. Swab samples of cutting surfaces used by seven (13.5%) out of 52 butchers yielded C-R isolates. The positive butchers' samples were from three slaughterhouses.

Four (7.4%) out of 54 C-R isolates were positive for phenotypic ESBL production. The detection rate of ESBL-producing bacteria in all 303 samples was 1.3%. The ESBL-producing isolates were *Es. coli* (three isolates) and *K. pneumoniae* (one isolate). They all harbored $bla_{\text{C-TX-M-15}}$ ESBL gene variant as well as $bla_{\text{TEM-1}}$ penicillinase gene. In addition, the *K. pneumoniae* isolate possessed the bla_{SHV} gene but the ESBL status of this gene was not determined. The ESBL-producing isolates originated from the fecal samples collected from three different farms located in two cities: two farms in Ifo with one ESBL-producing *Es. coli* each, and one farm in Sagamu with one ESBL-producing *Es. coli* and one *K. pneumoniae*. All three ESBL-producing *Es. coli* isolates belonged to phylogenetic group A.

Antimicrobial susceptibility testing showed that C-R isolates were highly resistant to many of the antimicrobials tested. Among the 54 C-R isolates, 100% were resistant to trimethoprim, 96.3% to cefoxitin and streptomycin, 94.4% to kanamycin, 92.6% to amoxicillin/clavulanic acid and nalidixic acid, 87.0% to chloramphenicol, 81.5% to sulfamethoxazole, 77.8% to ceftazidime and tetracycline, 75.9% to amikacin, 74.1% to ciprofloxacin, and 37.0% to gentamicin (Table III). All the isolates displayed multidrug resistance with resistance to at least one representative of each of the seven tested classes of antimicrobials (β-lactam, aminoglycosides, phenicol, fluoro/quinolone, sulfonamide, folate inhibitor and tetracycline).

Knowledge, attitude and practices of pig farmers on antimicrobial usage

All the pig farms investigated reported to use regularly antimicrobials in pig production. Forty-eight (80%) administered them on a monthly basis. Tetracycline was the most frequently used and was administered in the injectable form as oxytetracycline Long Acting (200 mg/ml). It was also frequently administered in oral form in combination with multivitamins as anti-stress agent. Other antimicrobials administered were ampicillin (35% of farmers), amoxicillin (33%), ciprofloxacin (20%), and streptomycin and penicillin (18%) (Table IV). The cephalosporins were among the least administered antimicrobials (3.3%). Most farmers (91.7%) administered antimicrobials mainly for the prevention of infections. Only five (8.3%) administered them principally for growth promotion. Antimicrobials such as chloramphenicol, gentamicin, ciprofloxacin, enrofloxacin and the cephalosporins were used purposefully for the treatment of infections. Only 18 (30.0%) engaged the services of veterinarians in disease management.

According to the farmers, the most commonly encountered diseases were parasitism (96.7%), diarrhea (83.3%), respiratory tract infections (70.0%), nutritional deficiencies and malnutrition (70.0%), and skin infections (66.7%). Only very few farmers (13.3%) practiced routine vaccination for disease prevention. Many farmers (63.3%) reported that they would discontinue the use of an antimicrobial without completing the recommended course once the clinical signs had resolved. Forty-four (73.3%) would increase the dose of an antimicrobial above the recommended dose if symptoms failed to abate within three days. Forty-one (68.3%) would change the type of antimicrobial or combine different antimicrobials in the presence of a refractory infection. All the farmers used antimicrobials without microbiological diagnosis nor antimicrobial susceptibility testing. Most of them (88.3%) were aware of the importance of observing a withdrawal period following antimicrobial therapy before slaughter of pigs. However, only 16 (26.7%) observed a withdrawal period before slaughtering pigs for human consumption. In case of antimicrobial resistant infection, most farmers (73.3%) disregarded observing the withdrawal period and slaughtered or sold sick pigs that were on antimicrobial treatment in order to minimize loss. All of them reported that they had ready access to antimicrobial and had never been asked to provide a prescription before purchasing them. Many of them (65.0%) agreed that pigs and pig products could serve as reservoirs and vehicles for the transmission of zoonotic pathogens including antimicrobial-resistant bacteria to humans. However, only 29 (48.3%) believed that

Table III

Antimicrobial resistance rate of cefotaxime-resistant Enterobacteriaceae isolated along the pig-production chain, Ogun State, Nigeria

Antimicrobial	*Escherichia coli* (n = 22)	*Klebsiella* spp. (n = 17)	*Enterobacter aerogenes* (n = 10)	*Citrobacter freundii* (n = 5)	All isolates (n = 54)
Chloramphenicol	86.4	76.5	100	100	87.0
Ciprofloxacin	59.1	82.5	80.0	100	74.1
Ceftazidime	68.2	94.1	60.0	100	77.8
Amoxicillin/clavulanic	100	94.1	80.0	80.0	92.6
Cefoxitin	100	94.1	100	80.0	96.3
Gentamycin	40.9	17.6	50.0	60.0	37.0
Tetracycline	77.3	76.5	80.0	80.0	77.8
Streptomycin	100	100	90.0	80.0	96.3
Amikacin	72.7	70.6	90.0	80.0	75.9
Nalidixic acid	100	82.5	100	80.0	92.6
Kanamycin	100	82.5	100	100	94.4
Trimethoprim	100	100	100	100	100
Sulfamethoxazole	72.7	76.5	100	100	81.5

Table IV

Commonly used antimicrobials in farms, Ogun State, Nigeria

Antimicrobial	Farm (%)	Antimicrobial	Farm (%)
Tetracycline	52 (86.7)	Sulfamethoxazole/ trimethoprim	5 (8.3)
Ampicillin	21 (35.0)	Chloramphenicol	3 (5.0)
Amoxicillin	20 (33.3)	Kanamycin	2 (3.3)
Ciprofloxacin	12 (20.0)	Ceftiofur	2 (3.3)
Enrofloxacin	12 (20.0)	Cefotaxime	2 (3.3)
Penicillin	11 (18.3)	Cefoxitin	2 (3.3)
Streptomycin	11 (18.3)	Amikacin	0 (0.0)
Gentamicin	6 (10.0)	Nalidixic acid	0 (0.0)

administration of antimicrobials to their pigs could facilitate the development of antimicrobial-resistance bacteria in these animals.

■ DISCUSSION

Cefotaxime-resistant Enterobacteriaceae were detected in all sample categories along the pig-production chain except in ready-to-eat (fried) pork. The rate of detection of these organisms was particularly high in fresh and frozen pork. C-R organisms survived in frozen meat but were destroyed by frying. Thus, pig production may be important in the dissemination of C-R Enterobacteriaceae in the study area, and fresh and frozen pork represent major vehicles for possible transmission of the organisms to humans. The detection of C-R organisms in swabs of cutting surfaces of knives and butchers' tables during slaughter suggests that these tools may play important roles in the contamination of meat thereby aiding the spread of the microbes from original animal host through meat at slaughterhouses and markets. The higher detection of the organisms in pork across slaughterhouses and retail shops than in on-farm pigs showed that contamination during processing at slaughterhouses and during marketing is critical for the dissemination and possible zoonotic transmission. Pigs from a small number of isolated farms in remote regions may harbor C-R bacteria but these bacteria may reach many people within and outside the originating farms through contaminated meat due to unhygienic practices during slaughtering, processing and marketing. It is important to note that the C-R bacteria identified in meat and cutting surfaces may not necessarily originate from pigs but could be from environmental and possibly human sources during handling.

This study also showed that different species of Enterobacteriaceae possess C-R traits. This is in agreement with previous reports where many different species of Enterobacteriaceae were found to be resistant to cefotaxime (Jean et al., 2002). Although not all the Enterobacteriaceae species encountered in this study are considered major pathogens, *Es. coli* and *K. pneumoniae* are known to cause significant morbidity in humans (Jean et al., 2002; Harris et al., 2015). *En. aerogenes* and *C. freundii* are also associated with opportunistic infections with various forms of complications (Gajdács and Urbán, 2019). Cefotaxime resistance could be due to the production of ESBL $_{CTX-M}$ type enzymes that hydrolyze and inactive third and fourth generation cephalosporins (Bauernfeind et al., 1990; Zhao and Hu, 2013). In this study, only four out of 54 C-R isolates were ESBL producers. These four isolates possessed $bla_{CTX-M-15}$ ESBL gene variant which encodes a very potent extended-spectrum cefotaximase. Moreover, only fecal samples from live pigs were positive for ESBL-producing bacteria. Thus, this study showed that the rate of detection of ESBL-producing bacteria was low in pigs in the area. Nevertheless, the detection of ESBL-producing bacteria in pigs is of public health significance. It shows that pigs are reservoirs and potential sources of ESBL-producing bacteria that could be transmitted to humans. The non-detection of ESBL-producing bacteria in other sample types could be a reflection of the low incidence in pigs in such a way that the chances of contamination from pig sources and subsequent detection in pork and other sources along the pig-production chain are limited.

The 1.3% ESBL-detection rate observed in our study is similar to that of 2.0% detected in pigs from Nsukka, Nigeria (Chah et al., 2018), but far lower than that of 51.1% detected in pigs from Thailand (Nuangmek et al., 2018). High detection of ESBL-producing bacteria has been linked with the administration of third and fourth generation cephalosporins in farm animals (Hammerum et al., 2014). However, third and fourth generation cephalosporins are rarely used in livestock production (including pig production) in Nigeria (Ojo et al., 2016a). The $bla_{CTX-M-15}$ is the predominant ESBL-gene variant in Nigeria and has been reported from different animal sources (Ojo et al., 2016b; Okpara et al., 2018) and from clinical isolates in humans (Aibinu et al., 2012). The $bla_{CTX-M-15}$ has global spread and is the most commonly identified ESBL-gene variant in human clinical conditions. The non-ESBL C-R bacteria encountered in this study may possess resistance mechanisms other than ESBL production. Overexpression of inducible chromosomally expressed AmpC β-lactamase could lead to cefotaxime resistance in Enterobacteriaceae (Jacoby, 2009). Studies have also shown that alteration of the outer membrane protein in Enterobacteriaceae could be responsible for resistance to β-lactams and other antimicrobials (Ojo et al., 2016b). This study did not investigate the other possible resistance mechanisms in the non-ESBL C-R isolates. ESBL genes are often located in transmissible mobile genetic elements especially plasmids, which could be shared among enteric bacteria by horizontally gene transfer thereby widening the niche of ESBL production in pathogenic and commensal bacteria populations. All ESBL-producing *Es. coli* isolates in our study belonged to phylogenetic group A. In an earlier study in the study area, most of ESBL-producing *Es. coli* that harbored $bla_{CTX-M-15}$ belonged to phylogenetic group A (Okpara et al., 2018). *Es. coli* strains in phylogroup A are predominantly commensal (Duriez et al., 2001). However, members of phylogenetic group A have been associated with extraintestinal infections in humans (Chakraborty et al., 2015).

The present study showed that C-R isolates were also resistant to many of the tested antimicrobials. The isolates were resistant to representative members of the following antimicrobial classes: β-lactams, aminoglycosides, phenicols, fluoro/quinolones, sulfonamides, folate inhibitors and tetracyclines. The high level of multidrug resistance observed is similar to the reports of other authors where C-R bacteria were shown to be multidrug resistant (Okpara et al., 2018; Fukuda et al., 2018). Earlier studies have reported a high level of multidrug-resistant Enterobacteriaceae in various animal species from the study area and elsewhere in Nigeria (Ojo et al., 2012; Oloso et al., 2018). The use of antimicrobials in animal production can select for resistant strains and facilitate their proliferation while eliminating competing susceptible bacteria. The selection and proliferation of antimicrobial-resistant bacteria in the presence of antimicrobial usage aided with unhygienic practices on farms and in slaughterhouses promote widespread dissemination of these bacteria in farm animals, environment and animal foods. The present study showed that cephalosporins including cefotaxime and ceftiofur were used in pig production albeit at a lower level compared to other antimicrobials. Cephalosporins were among the least used antimicrobials in pig production in the study area, whereas tetracycline was the most commonly used, as reported in other studies in Nigeria (Ojo et al. 2016a). Notwithstanding the low level of cephalosporins usage in pigs,

exposure of gut microflora to other antimicrobials could coselect for cefotaxime resistance in the exposed bacteria. Pigs can also acquire C-R bacteria from other sources including feed and water sources. ESBL-producing Enterobacteriaceae were detected in the two farms that reported the use of cephalosporins and in another farm where cephalosporins were not used. The emergence of ESBL-producing bacteria in humans and animals has been linked with the use of cephalosporins (Greko et al., 2009). Farmers in this study knew that pigs could serve as reservoirs of pathogens transmissible to humans but were ignorant of the fact that the use of antimicrobials in their animals could promote the emergence of antimicrobial resistant strains. This signifies an inadequate level of awareness among pig farmers on factors that contribute to the emergence of antimicrobial resistance. Poor regulation of the sale of antimicrobials may also encourage the use of antimicrobials in pigs because farmers reported that they were never asked to provide a prescription before their purchase. Earlier studies also report the poor sale regulation and easy access to antimicrobials by animal producers in Nigeria (Ojo et al., 2017).

■ CONCLUSION

This study revealed a widespread distribution of C-R Enterobacteriaceae (of multidrug-resistance status) in the pig-production chain in Ogun State, Nigeria. Slaughterhouses and meat represent major points of contamination and vehicles for the spread of C-R bacteria. This study also showed that pigs harbor ESBL-producing *Es. coli* and *K. pneumoniae* that possess the $bla_{\text{CTX-M-15}}$ gene. There is a need to raise the level of awareness on the role of antimicrobial usage in the development of antimicrobial resistance because most pig farmers did not know that the use of antimicrobials contributes to the emergence of antimicrobial resistance. Strict monitoring of antimicrobial sales and compliance with existing regulatory policies guiding the use and distribution of antimicrobials could reduce overdependence on antimicrobials in animal production. These are desirable steps in the preservation of the efficacy and benefits of antimicrobials in medical and veterinary practices.

Author contributions statement

OEO, MAD and EAA conceptualized and designed the study. OEO and AMI participated in samples collection and microbiological analyses. OEO was responsible for the molecular characterization of isolates. OEO and AMI participated in data analyses and preparation of the manuscript. JOH, MAD and EAA critically reviewed the manuscript.

Conflicts of interest

The study was carried out without any conflict of interest.

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Résumé

Ojo O.E., Iledare A.M., Amosun E.A., Hassan J.O., Dipeolu M.A. Usage d'antimicrobiens et détection d'entérobactéries résistantes à la céfotaxime dans la chaîne de production porcine, Etat d'Ogun, Nigeria

Le céfotaxime appartient au groupe des céphalosporines de troisième génération (3GC) qui sont classées comme des agents antimicrobiens d'importance critique pour le traitement des infections chez l'homme. La recrudescence de l'incidence des bactéries résistantes à la céfotaxime d'origine animale est importante pour la santé publique mondiale. Cette étude a porté sur la présence d'entérobactéries résistantes à la céfotaxime dans la chaîne de production porcine de l'état d'Ogun, au Nigeria, et a examiné des isolats résistants à la céfotaxime pour la production de β-lactamase (ESBL) à spectre étendu. Les connaissances, l'attitude et les pratiques des éleveurs de porcs concernant l'utilisation des antimicrobiens ont également été étudiées. Des bactéries résistantes à la céfotaxime ont été détectées dans 54 (17,8 %) des 303 échantillons. Les isolats résistants à la céfotaxime ont été identifiés comme étant *Escherichia coli* (n = 22), *Klebsiella* spp. (n = 17), *Enterobacter aerogenes* (n = 10) et *Citrobacter freundii* (n = 5). Les organismes étaient présents dans les fèces des porcs d'élevage (15/109 ; 13,7%), la viande de porc fraîche dans les abattoirs (19/40 ; 47,5%), la viande de porc congelée dans les magasins

Resumen

Ojo O.E., Iledare A.M., Amosun E.A., Hassan J.O., Dipeolu M.A. Uso de antimicrobianos y detección de Enterobacteriaceae resistente a la cefatoxima en la cadena de producción del cerdo, estado Ogun, Nigeria

La cefotaxima pertenece al grupo de antimicrobianos de las cefalosporinas de tercera generación, que están clasificados como críticos para el tratamiento de infecciones en humanos. El aumento en la incidencia de bacterias resistentes a la cefotaxima (C-R) con origen animal es importante para la salud pública mundial. Este estudio investigó la presencia de Enterobacteriacea C-R en la cadena de producción porcina en el estado de Ogun, Nigeria, y examinó los aislamientos de C-R para la producción de β-lactamasa de espectro extendido (BLEE). Se estudiaron también los conocimientos, la actitud y las prácticas de los criadores de cerdos, referentes al uso de antimicrobianos. Se detectaron bacterias C-R en 54 (17,8%) de 303 muestras. Los aislamientos de C-R se identificaron como *Escherichia coli* (n = 22), *Klebsiella* spp. (n = 17), *Enterobacter aerogenes* (n = 10) y *Citrobacter freundii* (n = 5). Los organismos estaban presentes en las heces de cerdos de finca (15/109; 13,7%), carne de cerdo fresca en mataderos (19/40; 47,5%), carne de cerdo congelada en tiendas minoristas (7/28; 25,0%), superficies de corte de utensilios de carnicero (7/52; 13,5%) y agua efluente de mataderos (6/41; 14,6%). No

de détail (7/28 ; 25,0%), les surfaces de coupe des outils de boucherie (7/52 ; 13,5%) et les eaux usées des abattoirs (6/41 ; 14,6%). Aucune bactérie résistante à la céfotaxime n'a été détectée dans la viande de porc prête à consommer. Trois isolats de *Es. coli* et un de *K. pneumoniae* étaient producteurs d'ESBL et possédaient la variante du gène ESBL $bla_{CTX\text{-}M\text{-}15}$. Les *Es. coli* producteurs d'ESBL appartenaient au groupe phylogénétique A. Tous les isolats résistants à la céfotaxime étaient multirésistants aux médicaments et résistants à plus de trois agents antimicrobiens de différentes classes d'antimicrobiens. La tétracycline, l'ampicilline, l'amoxicilline, la ciprofloxacine et l'enrofloxacine faisaient partie des agents antimicrobiens couramment utilisés dans la production porcine, alors que les céphalosporines étaient rarement utilisées. Les éleveurs savaient que les porcs pouvaient servir de réservoirs de bactéries pathogènes transmissibles à l'homme. Cependant, ils ne savaient pas que l'utilisation d'antimicrobiens dans la production porcine pouvait entraîner le développement et la prolifération de bactéries résistantes aux antimicrobiens chez les porcs. Des efforts devraient être faits pour améliorer la sensibilisation des éleveurs sur le rôle de l'utilisation des antimicrobiens dans l'émergence et la diffusion des bactéries résistantes aux antimicrobiens dans la production animale.

Mots-clés: porcin, *Escherichia coli, Klebsiella pneumoniae*, Enterobacteriaceae, résistance aux antimicrobiens, céfotaxime, Nigeria

se detectó bacteria C-R en carne de cerdo lista para el consumo. Tres aislamientos de *Es. coli* y uno de *K. pneumoniae* fueron productores de BLEE y presentaban la variante del gen $bla_{CTX\text{-}M\text{-}15}$ BLEE. La *Es. coli* productora de BLEE perteneció al grupo filogenético A. Todos los aislamientos C-R fueron resistentes a más de tres antimicrobianos de diferentes clases de antimicrobianos. La tetraciclina, ampicilina, amoxicilina, ciprofloxacina y enrofloxacina se encontraron entre los antimicrobianos comúnmente utilizados en la producción porcina, mientras que las cefalosporinas se usaron rara vez. Los finqueros sabían que los cerdos podían servir como reservorios de bacterias patógenas transmisibles a los humanos. Sin embargo, no sabían que el uso de antimicrobianos en la producción porcina podría conducir al desarrollo y proliferación de bacterias resistentes a los antimicrobianos en los cerdos. Deben realizarse esfuerzos para mejorar la conciencia de los agricultores sobre el papel del uso de antimicrobianos en la aparición y diseminación de bacterias resistentes a los antimicrobianos en la producción animal.

Palabras clave: cerdo, *Escherichia coli*, *Klebsiella pneumoniae*, Enterobacteriaceae, resistencia a los antimicrobianos, cefotaxime, Nigeria