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Antimicrobial usage and presence of extended-spectrum β-lactamase-producing Enterobacteriaceae in animal-rearing households of selected rural and peri-urban communities

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ABSTRACT

This study examined socioeconomic and cultural factors relating to animal husbandry, antimicrobial usage and household hygiene in 320 animal-keeping households of 16 rural and peri-urban communities of Ogun State, Nigeria. The occurrence of extended-spectrum β-lactamase-producing Enterobacteriaceae in 457 samples from animal and environmental sources within the households was investigated. Chickens (41.6%), goats (35.3%), dogs (33.8%) and sheep (14.4%) were the most common household animals. Animals were reared mainly for income generation (73.9%) and for household consumption (18.3%). They were reared predominantly (60.2%–100%) under the extensive system with unrestricted access to human space, cooking utensils and foods. Households were assessed as having good (59.4%), fair (22.2%) and poor (18.4%) hygiene. The rate of household non-prescriptional antimicrobial usage was 69.4% in humans and 60.6% in animals. Overall, ESBL-producing Enterobacteriaceae were detected in 53 (11.6%) of 457 samples. The ESBL-producing isolates were identified as *Escherichia coli* (n = 49) and *Klebsiella pneumoniae* (n = 4). They harboured the ESBL gene variants $bla_{CTX\text{-}M\text{-}15}$ (n = 49), $bla_{CTX\text{-}M\text{-}14}$ (n = 2), $bla_{CTX\text{-}M\text{-}27}$ (n = 1) or $bla_{CTX\text{-}M\text{-}55}$ (n = 1). Forty-eight ESBL-producing *E. coli* were assigned into phylogenetic groups A (n = 17), B1 (n = 14), D (n = 13) and F (n = 4). All ESBL-producing isolates demonstrated multidrug resistance to antimicrobial agents belonging to at least three different classes of antimicrobials. Poor regulation of antimicrobial marketing and inadequate access to veterinary care contributed to non-prescriptional use of antimicrobials in humans and animals. Free-range household animals harboured ESBL-producing bacteria and may facilitate the dispersal of the organisms within the community.

1. Introduction

Household animal rearing is a common practice to augment family income and serve as ready source of animal protein in many rural and peri-urban areas of Nigeria as well as in other developing countries (Pica-Ciamarra et al., 2011; Owolade et al., 2013). With household animal rearing, humans live in very close contact with animals under compromised hygiene conditions (Rwego et al., 2008). In most African communities, household livestock is characterised by animals having unrestricted access to human dwellings, cooking utensils, drinking water and food meant for human consumption (Schriewer et al., 2015; Ercumen et al., 2017). Direct and indirect contact with animals and contaminated environment facilitate easy transmission of microorganisms between humans and animals (Herrero et al., 2012). A great proportion of household animals in the rural and peri-urban settings are reared under the extensive and semi-intensive systems of management where animals are exposed to diverse populations of microorganisms in the environment (Adesehinwa et al., 2004; Maass et al., 2012; Malatji et al., 2016). These free-roaming animals also contribute to environmental contamination by shedding of microorganisms in the environment thus contributing to widespread dissemination of microorganisms in the community (Ogden et al., 2009; Herrero et al., 2012).

. In Nigeria, livestock are kept predominantly under the subsistence, small-scale extensive management system with low input from farmers (Bourn et al., 1994; FAO, 2005; Pica-Ciamarra et al., 2011). Most of these farmers live in the rural areas. The intensively managed production system accounts for only 11% of total chicken population; 3% of the pig population; and less than 1% of the cattle, sheep and goat

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populations (Bourn et al., 1994). Administration of antimicrobial agents is very crucial to the sustainability of rural and peri-urban household animal rearing due to the high level of exposure to infectious agents (Ojo et al., 2016a). Overdependence on antimicrobial agents and misuse are the inevitable outcome of unrestricted access and poor regulation of the use of antimicrobial agents. The β-lactam antibiotics are among the most commonly used antibiotics in veterinary and human medicine. These antibiotics are relatively cheap and safe with very low toxicity compared to other classes of drugs. The emergence and dissemination of extended spectrum β-lactamase (ESBL)-producing bacteria with resistance to penicillins and new generation cephalosporins have reduced the benefits derivable from the use of the β-lactam antibiotics in the treatment of human and animal diseases. Due to the close contact between animals and humans in households, there could be an easy exchange of commensal as well as pathogenic ESBL-producing bacteria.

The present study investigated antimicrobial usage, sociocultural and economic characteristics as well as the presence of ESBL-producing Enterobacteriaceae in animal-keeping households in rural and peri-urban communities of Ogun State, Nigeria. It also examined household hygiene and the occurrence of diseases symptoms (especially diarrhoea) in humans cohabiting with animals within the same households.

2. Materials and methods

2.1. Study area

This study was carried out in selected rural and peri-urban communities of Ogun State. Ogun State is located in the Southwestern region of Nigeria (Fig. 1). The main occupation of the people in the State is farming with high preponderance of commercial livestock production and subsistence household animal rearing. The State is divided into four geopolitical zones, which are Egba, Ijebu, Remo and Yewa zones. Each geopolitical zone consisted of people of similar cultural and ethnic background. All the four zones were included in the study. The sampling sites were identified and categorized into rural and peri-urban communities. Categorization was based on population size, major commercial activities, major occupations and means of livelihood, lifestyle, availability of social amenities, nearness to major cities/metropolitan centres and perceived level of civilization. In the context of this study, a rural community is an area of less than 20,000 inhabitants predominantly of agrarian lifestyle, usually characterised by poor social amenities and poor living standard (Agbodike, 2010). A peri-urban community is a transition area between rural and urban that is adjacent to an urban centre and with ready means of transportation to the urban centre (Mandere et al., 2010). In peri-urban community, there is a clash of interest between traditional agricultural activities and alternative economic, residential and recreational activities (Mandere et al., 2010). A community is a group of people of common sociocultural characteristics living together in a clearly demarcated geographical location identifiable by a name and separated from other groups. Sixteen communities, comprising eight rural and eight peri-urban communities were included in the investigation. In each of the four zones, two rural and two peri-urban communities were included in the study. The specific communities were chosen by balloting. In balloting, names of rural and peri-urban communities in each zone were written in separate pieces of paper and put in two boxes; one for rural and the other for peri-urban. The pieces of paper in each box were shuffled and two pieces were blindly picked out of the box. Thus, four names (including two rural and two peri-urban) were picked from the two boxes for each zone. The communities picked were included in the study. Within each community, households were selected based on informed consent. Every fifth animal-keeping household in each community was approached for informed consent until the required number of household was completed. Only animal-keeping households were approached to obtain informed consent for participation in the study. In each household, the head of the household was informed about the study in the local language.

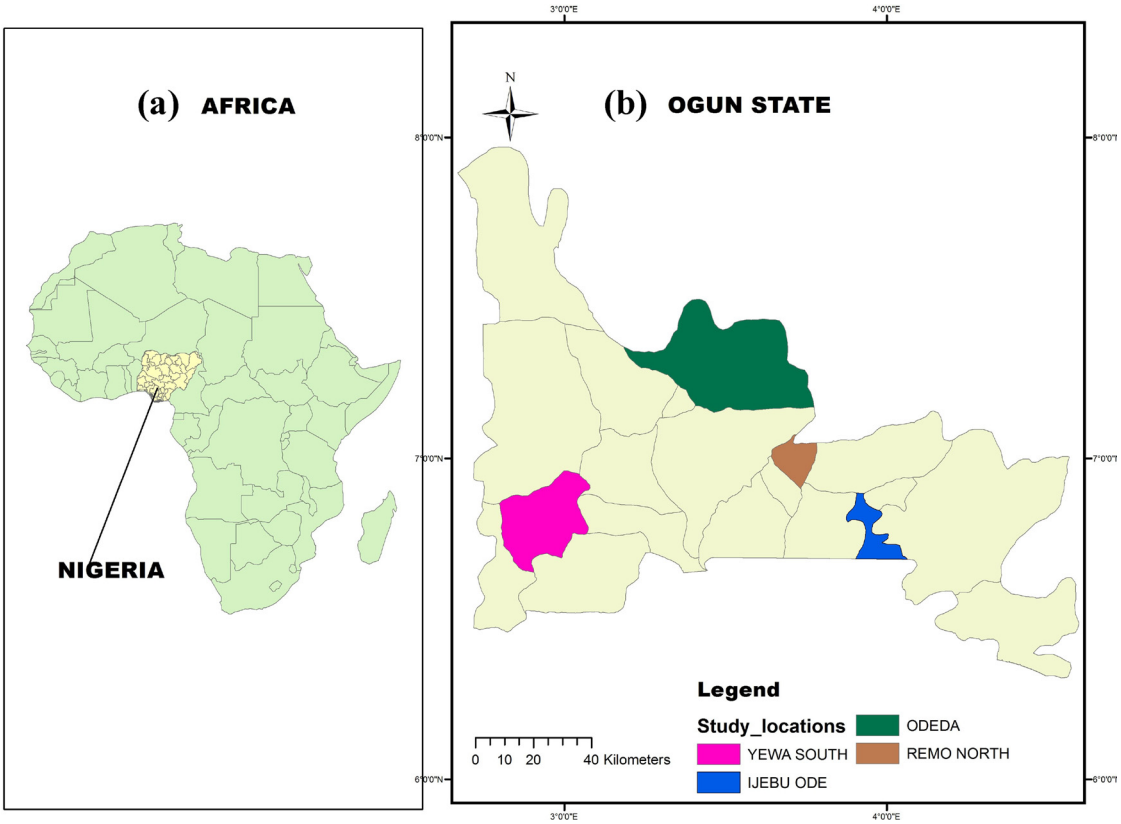

Fig. 1. (a) Location of Nigeria in the African continent and (b) the four Local Government Areas (LGAs) of the Ogun State which were sampled in this study.

2.2. Collection of socioeconomic/cultural samples

Focus group discussions (FGD), in-depth interviews, semi-structured questionnaire and observational studies were employed to collect data on knowledge, attitude and practices of animal keepers in relation to antimicrobial usage, household hygiene, disease symptoms, and factors that favour zoonotic transmission of bacteria between humans and animals within animal-keeping households. Available members of the household were involved in a focus group discussion before conducting an in-depth interview with the head of the household or his/her delegate. The information collected were grouped into eight categories as follows: household characteristics/demographic data, animal management practices, household hygiene, consumption of animal products, accessibility to medical and veterinary care, prevailing health issues/challenges, antimicrobial usage pattern (including knowledge, practices and attitude on antimicrobial usage and knowledge of zoonoses) and experience of antimicrobial therapeutic failure (Supplementary document S1). In total, 320 households were investigated from the 16 communities.

2.3. Analysis of socioeconomic/cultural data

To determine the level of hygiene within households, the household heads or the owners of animals within households were asked questions about practices on personal hygiene while handling animals. The hygiene level was score based on eight questions on Likert scale of four (Sullivan and Artino et al., 2013). Points obtainable for each choice ranged from 0 to 3 (never = 0, rarely = 1, often = 2 and always = 3) with a maximum of 24 obtainable points. Households where the respondents scored 12 points or less were categorised as having poor level of hygiene, those with 13–18 points were categorised as having fair level of hygiene and those with 19–24 points were categorised as having good level of hygiene. To assess the level of knowledge in relation to antimicrobial usage and animal diseases, responses to a set of 25 questions were used for scoring. Among them, correct answers to twenty-two questions were rated with either zero or one point, two questions had 0–2.5 points while one question had 0–3 points. Altogether, the maximum obtainable points for all the 25 knowledge-based questions was 30. Based on the scores obtained, households were rated as having poor ($\leq$15 points), fair (16–20 points) or good knowledge ($\geq$20 points).

2.4. Statistical analysis

The data from the questionnaire was assessed for normality using a skewness test. Continuous variables were expressed in terms of means and standard deviations while categorical variables were presented in frequencies and percentages. Associations between the hygiene scores (dependent variable), demographic data (independent variables) and source of information (independent variables) were assessed with a negative binomial regression model. Spearman rho was used to determine correlation of the presence of ESBL-producing bacteria versus the hygiene score, antimicrobial usage in humans and animals, common diseases in humans and animals, animal access to human habitations as well as the presence of animal faeces in the environment in each of the communities investigated. The prevalence of ESBL-producing bacteria among the different animal species was compared using Chi-square test and P values of $^{<}$0.05 were considered statistically significant. All data were analysed using Statistical Package for the Social Sciences software (SPSS version 16).

2.5. Collection of biological samples

Biological samples were collected alongside with the socioeconomic data from households. Biological samples included faecal samples from dogs (n = 108), goats (n = 113), chickens (n = 101), sheep (n = 46), pigs (n = 7), ducks (n = 5) and turkey (n = 9). Other were pigeons (n = 2), guinea fowl (n = 2), cat (n = 1), monkey (n = 1) and rabbit (n = 1). Faecal samples were collected directly from the rectum of animals within the households using sterile swabs. Where possible, samples were collected from all animal species within a household. However, only one sample represented each species in every household. The swabs were inoculated directly into 9 mL of buffered peptone water (BPW). In addition, environmental samples including swabs of surfaces of shared human/animal spaces (n = 15), samples of human foods (n = 2), drinking water (n = 15), animal feeds (n = 9) as well as human (n = 1) and animal excreta around houses (n = 18) were collected. Five grams (solid samples) or one millilitre (liquid samples) of sample was inoculated directly into nine millilitre of sterile buffered peptone water (BPW). Animal and human excreta as well as swabs of surfaces were collected based on observed environmental contamination while feed and water samples were obtained based on availability within the household premises. The inoculated samples were appropriately labelled and preserved in ice-pack for transportation to the laboratory. In total, 457 samples were collected from 320 households.

Both socioeconomic data for assessment of antimicrobial usage and biological samples for detection of ESBL-producing bacteria were collected simultaneously during the months of September to December 2016.

2.6. Selective isolation of putative ESBL-producing bacteria from biological samples and confirmatory tests for ESBL-producing bacteria

In the laboratory, the aforementioned samples in BPW were incubated at 37 °C overnight for pre-enrichment (Jazmati et al., 2016). A loopful of the BPW culture was inoculated onto MacConkey agar supplemented with ampicillin (100 mg/L; Mac-AMP_{100}). Inoculated plates were incubated at 37 °C for 18–24 h and examined for bacterial growth. Both lactose fermenters and non-lactose fermenters on Mac-AMP_{100} were selected for further screening on MacConkey agar containing cefotaxime supplement (1 mg/L; Mac-CTX_1). Isolates that grew on both selective agar media were suspected as ESBL-producers. These were preserved in tryptic soy broth with glycerol and kept at −20 °C for further studies.

Isolates recovered following the selective culture of samples on Mac-AMP_{100} and Mac-CTX_1 were tested for phenotypic ESBL-production using the cefpodoxime/cefpodoxime-clavulanic combination discs test kit (Oxoid DD0029) according to the manufacturer's instruction and in line with the recommendations of Clinical and Laboratory Standards Institute (CLSI, 2015).

2.7. Bacterial identification

Initial bacterial identification was based on cultural characteristics of lactose and non-lactose fermentation on MacConkey agar. Further identification of phenotypic ESBL-producers into genera and species was based on biochemical characterization using commercial biochemical kit for the identification of Gram negative bacteria (Microbact GNB 24E, Oxoid^{R}, Basingstoke, UK) and the MALDI-TOF MS technique using the MALDI Microflex LT (Bruker Daltonics, Bremen, Germany).

2.8. Phenotypic antimicrobial susceptibility testing

ESBL-producing isolates were tested for susceptibility to ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), kanamycin (30 µg), gentamicin (10 µg), streptomycin (10 µg), amikacin (30 µg), tetracycline (30 µg), chloramphenicol (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), trimethoprim (5 µg), compound sulfonamides (300 µg) and sulfamethoxazole/trimethoprim (25 µg) by the disk diffusion method. Antimicrobial susceptibility test was performed on Mueller-Hinton agar according to the Clinical Laboratory Standards Institute document VET01-A4 (Clinical and Laboratory Standards Institute (CLSI,

2013). *Escherichia coli* ATCC 25922 was included as quality control strain.

2.9. Detection of ESBL genes and determination of E. coli phylogenetic groups

Genomic DNA was extracted from overnight culture of bacterial cells grown in Brain Heart Infusion (BHI) broth by thermo-lysis as previously described (Ojo et al., 2016b). The concentration of extracted DNA was quantified by spectrophotometry (Thermo Scientific NanoDrop^R 1000 spectrophotometer). A final DNA working concentration of 100 ng/μL was used for all PCR protocols. Isolates were screened for the initial detection of $bla_{\text{CTX-M-group1}}$, $bla_{\text{CTX-M-group9}}$, bla_{SHV} and bla_{TEM} genes (Olesen et al., 2004; Gröbner et al., 2009; Cullik et al., 2010) and were further subjected to PCR assays for sequencing of the whole bla_{SHV}, $bla_{\text{CTX-M-group1}}$ and $bla_{\text{CTX-M-group9}}$ genes (Olesen et al., 2004; Carattoli et al., 2008; Gröbner et al., 2009). In the presence of the genes $bla_{\text{CTX-M-group1}}$ or $bla_{\text{CTX-M-group9}}$, the bla_{TEM} and bla_{SHV} genes were not sequenced. Gene sequencing was done by LGC Genomic, Berlin, Germany. The nucleotide sequences were analysed using the bioinformatics software Geneious® 10.1.3 (Biomatters Limited, New Zealand). To determine the ESBL gene variants, analysed sequences were subjected to comparisons with reference sequences assigned at the Lahey Clinic website (http://www.lahey.org) and deposited at the National Center for Biotechnology (NCBI) website by using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

E. coli isolates were assigned to one of seven *E. coli sensu stricto* phylogenetic groups (A, B1, B2, C, D, E, and F) using a PCR-based method described by Clermont and colleagues (Clermont et al., 2013).

3. Results

3.1. Personal attributes of household animal keepers

The demographic and socioeconomic characteristics of household animal keepers showed that out of the 320 respondents (one person for each animal-keeping household), 173 (54.1%) were male and 147 (45.9%) were female indicating a fair representation of both sexes. Concerning the age, about 70% of all the 320 household animal keepers interviewed were within the working age group of 31–60 years. The respondents were almost of homogeneous ethnicity being predominantly (88.1%) of Yoruba descent and were mostly married (66.2%). Some of the respondents had no formal education (24.7%), others had a primary (19.7%) only or with secondary (37.2%) education. Only few respondents (18.4%) had post-secondary education. The most common primary occupations among the respondents were trading (32.2%), artisan (20.6%) and farming (20.0%). Other primary occupations were civil service (5.9%), teaching (9.4%), food processing (3.1%), transport business (5.0%) and religious leaders (3.8%). Concerning monthly income, 36.6% of the respondents earned less than the national minimum wage of 18,000 naira (which corresponds to €50) monthly while 42.8% earned above the minimum wage. Sixty-six (20.6%) respondents did not declare their monthly income. Only 24 (7.5%) indicated to be members of cooperative groups and trade/social associations.

3.2. Types of animals reared in households

The most commonly reared animals within household settings in the study areas were chickens (41.6%), goats (35.3%), dogs (33.8%), sheep (14.4%) and cattle (5.0%) (Table 1). Animals were reared predominantly under the extensive system of management and for economic purposes to generate income (Table 1). Animals were also reared as sources of food for household consumption, to offer sacrifices to deities, gifts for families and friends and for companionship. In the extensive system of management, animals were left to freely roam about and fend for themselves with only minimal input from the owners. Free-range scavenging animals in the extensive management system had access to human space, cooking utensils and foods thereby contributing to environmental pollution. Under the semi-intensive management system, animals were tethered within a grazing area usually near the household or allowed to roam about in search of food during the day but led to makeshift shelters at night. There were occasional veterinary care and supplementary feeding to augment nutritional supply. Intensive management entails complete confinement of animals within a pen with provision of veterinary care, feed and water.

3.3. Waste disposal methods and availability of toilet facilities in households

Waste disposal methods were generally inadequate. Two hundred and twenty-four households (70.0%) dumped wastes in open spaces near the house, 36 (11.8%) dumped their wastes in communal dunghills, while 60 (18.8%) utilised the services of government or private waste collectors that regularly collect waste from residential buildings. Available toilet facilities for household use included water closets (36.9%), pit latrines (51.2%) and communal toilets (2.5%). Thirty households (9.4%) did not have toilet facilities but members defaecated in bushes near their houses.

3.4. Personal hygiene within households

Household members practiced some form of personal hygiene measures such as regular hand washing with soap after handling animals (55.6%), after sneezing (55.6%), after visiting the toilet (69.4%), before and after cooking (56.9%) and after slaughtering animals (65.3%). However, only 78 (24.4%) households regularly cleaned animal pens. Overall, 190 (59.4%) households had good, 71 (22.2%) had fair and 59 (18.4%) had poor standards of hygiene. The mean knowledge score on household hygiene was 15.27 ± 6.41 out of a maximum of 24 obtainable points. The hygiene score was significantly associated ($p \leq 0.01$) with Local Government Area (LGA), age, marital status, education, ethnicity and source of information. The strongest positive predictor of hygiene score was being from Yewa-South LGA followed by obtaining information on animal health from veterinary personnel (Supplementary document S2).

3.5. Household antimicrobial usage

Antimicrobial usage was common (100%) among human members of all the 320 animal-keeping households investigated. Antimicrobials were used in humans for the treatment (33.8%) and prevention (19.7%) of diseases or for both therapeutic and preventive purposes (46.5%). Commonly used antimicrobials in humans include ampicillin (38.4%), ampicillin/cloxacillin (37.5%), tetracycline (28.1%), penicillin (26.9%), amoxicillin (26.6%), trimethoprim/sulfamethoxazole (22.2%), chloramphenicol (10.9%) and ciprofloxacin (9.1%) (Table 2). Two hundred and twenty-two (69.4%) of 320 households indicated that they had purchased antimicrobials for use in humans without prescription from a physician. Antimicrobials were purchased based on the occurrence of familiar symptoms of diseases or past experience of drug efficacy when used in the treatment of disease symptoms. One hundred and sixty (50.0%) households would keep antimicrobials packages and labels for future need.

This study showed that 206 (64.4%) of 320 households administered antimicrobials to their animals. Antimicrobials were used for the prevention (25.0%) and treatment (23.1%) of diseases as well as for growth promotion (16.3%) purposes. The following are some of the antimicrobials administered to household animals: long acting oxytetracycline (10.0%), penicillin/streptomycin (7.8%), tetracycline (8.4%), ampicillin (6.9%) and ampicillin/cloxacillin (5.3%) (Table 2). One hundred and ninety-four (60.6%) of 320 households indicated that they used antimicrobials in animals without prescription by a

Table 1
Types of animals reared in households of rural and peri-urban communities of four Local Government Areas in the Ogun State, Nigeria.

Types of animals (No. of households)	Percentage of total household	System of management No. (%)			Reasons for rearing animals No. (%)		
		Extensive	Semi-intensive	Intensive	Economic	Family consumption	Other reasons
Chicken (133)	41.6	80 (60.2)	45 (33.8)	6 (4.5)	87 (65.4)	41 (30.8)	4 (3.0)
Goat (113)	35.3	79 (69.9)	33 (29.2)	1 (0.9)	89 (78.8)	19 (16.8)	5 (4.4)
Dog (108)	33.8	68 (62.9)	37 (34.3)	2 (1.9)	48 (44.4)	7 (6.5)	51 (47.2)
Sheep (46)	14.4	31 (67.4)	14 (30.4)	1 (2.2)	44 (95.7)	1 (2.2)	1 (2.2)
Cattle (16)	5	14 (87.5)	0	2 (12.5)	16 (100)	0	0
Turkey (9)	2.8	7 (77.8)	2 (22.2)	0	9 (100)	0	0
Pigs (7)	2.2	0	3 (42.9)	4 (57.1)	5 (71.4)	2 (28.6)	0
Duck (4)	1.3	4 (100)	0	0	3 (75.0)	1 (25.0)	0
Guinea fowl (2)	0.6	2 (100)	0	0	2 (100)	0	0
Pigeon (2)	0.6	2 (100)	0	0	0	0	2 (100)
Monkey (2)	0.6	0	0	2 (100)	0	0	2 (100)
Rabbit (1)	0.3	0	0	1 (100)	1 (100)	0	0
Parrot (1)	0.3	0	0	1 (100)	0	0	1 (100)
Catfish (1)	0.3	0	0	1 (100)	1 (100)	0	0
Cat (1)	0.3	1 (100)	0	0	0	0	1 (100)
Quail (1)	0.3	0	0	1 (100)	1 (100)	0	0

Table 2
Household antimicrobials usage in animals and humans in selected urban and peri-urban communities of four Local Government Areas in the Ogun State, Nigeria.

Antimicrobial agent	Number of households (%) in which antimicrobial agents were used in animals	Number of households (%) in which antimicrobial agents were used in humans
Tetracyclines		
Oxytetracycline long acting	32 (10)	–
Tetracycline	27 (8.4)	90 (28.1)
β-Lactams		
Ampicillin	22 (6.9)	123 (38.4)
Ampicillin/cloxacillin	17 (5.3)	121 (37.8)
Amoxicillin	12 (3.8)	85 (26.6)
Cephalosporins	5 (1.6)	12 (3.8)
Amoxycillin/clavulanic acid	5 (1.6)	20 (6.3)
Penicillin	9 (2.8)	86 (26.9)
β-Lactams/Aminoglycoside combination		
Penicillin/Streptomycin	25 (7.8)	–
Phenicols		
Chloramphenicol	5 (1.6)	35 (10.9)
Fluoroquinolones		
Norfloxacin	4 (1.3)	–
Flumequine	3 (0.9)	–
Enrofloxacin	7 (2.2)	–
Ciprofloxacin	6 (1.9)	29 (9.1)
Aminoglycosides		
Gentamicin	6 (1.9)	18 (5.6)
Neomycin	4 (1.3)	3 (0.9)
Streptomycin	3 (0.9)	15 (4.7)
Macrolide		
Erythromycin	3 (0.9)	4 (1.3)
Folic acid inhibitor and sulphonamides		
Trimethoprim/ sulphamethoxazole	3 (0.9)	71 (22.2)
Sulfonamides	3 (0.9)	–
Polymyxin		
Colistin	4 (1.3)	–
Nitrofurans		
Furazolidone	4 (1.3)	–

veterinarian. One hundred and seventy (53.1%) households agreed that prescription by a veterinarian is necessary before antimicrobial usage in animals while 150 (46.9%) think veterinary prescription is not necessary before antimicrobial usage.

The mean knowledge score on antimicrobial usage was 15.0 ± 5.0 out of 30 maximum obtainable points. The knowledge scores on antimicrobials and antimicrobial usage was significantly associated ($p \leq 0.01$) with Local Government Area, marital status and sources of information (Table 3). Livestock owners from Yewa South and Remo North LGAs or those from Odeda, Porogun, Ososa and Isiwo villages scored high on knowledge. Livestock owners who sourced information on animal health from internet, fully divorced or monogamous (if married), had a low knowledge score.

3.6. Household access to human and veterinary healthcare

Two hundred and sixty (81.3%) of 320 households indicated that there was at least a human medical centre/hospital within their vicinity of about 30 km while 286 (89.4%) indicated that there was at least one pharmacy/patent medicine store in their community. However, only 200 (62.5%) households would go to human medical centre/hospital for medical services. Sources of help for healthcare services included

Table 3
Predictors of knowledge score on antimicrobial usage identified by negative binomial regression analysis in a sample of 320 livestock owners in four selected Local Government Areas of the Ogun State.

Variables	Coefficient	*p*-Value	95% CI [a]	IRR [b]
Local Government Area				
Yewa South	0.922	< 0.001	0.519–1.325	2.515
Remo North	0.687	< 0.001	0.346–1.029	1.989
Villages				
Odeda	0.796	< 0.001	0.513–1.080	2.218
Porogun	0.703	< 0.001	0.405–1.001	2.020
Ososa	0.379	0.038	0.021–0.737	1.461
Isiwo	0.556	< 0.001	0.294–0.818	1.744
Marital Status				
Fully divorced	−0.440	0.007	−0.759 to 0.122	0.644
Married (monogamy)	−0.184	0.050	−0.369 to 0.000	0.832
Internet as a source of information for human health	0.857	0.034	0.065–1.648	2.355
Internet as a source of information for animal health	−0.840	0.012	−1.493 to 0.187	0.432

Note: More information on all the variables investigated can be found in the questionnaire available as supplementary document.
[a] CI: confidence interval.
[b] IRR: Incidence rate ratio.

patent medicine stores (21.9%), homemade remedies including herbal preparations (14.1%) and spiritual healers (1.8%). One hundred and one (31.6%) of the 320 households indicated that no human member of their household had sought or received medical care from a medical centre/hospital within the past five years.

Many of the households never (51.5%) or rarely (23.1%) have access to veterinary services for their animals. Two hundred and twenty-eight (71.3%) of the households indicated that they had no veterinary drug store in their community. When animals showed signs of illness, such sick animals were slaughtered and consumed by household members (20.6%), sold to butchers/meat processor for slaughter and the meat sold for public consumption (15.0%), treated with antimicrobials by household members without veterinary intervention (18.4%), treated with herbs and other homemade remedies (8.1%) or taken to a veterinary clinic (26.2%).

3.7. Disease occurrence in humans and animals

Commonly encountered disease symptoms in human members of households included fever (18.8%), headache (17.8%), diarrhoea (16.6%), general body pain (10.3%), joint pain (10.3%), abdominal cramps (9.1%), coughing with chest pain (7.8%) and backache (7.5%). Others were nasal discharges (4.1%), skin infections (3.8%), bloody diarrhoea (3.4%), eye infections (2.8%), ear infections (2.8%), and toothache (0.9%). At the time of sampling, 15 (4.7%) households indicated that there were ongoing cases of diarrhoea in at least one member of the household.

Common clinical signs of disease in animals include loss of appetite (9.4%), diarrhoea (22.5%), dullness (5.9%), nasal discharges (6.3%), wounds (7.5%), skin infections (5.3%), emaciation (2.5%), eye infections (6.3%) and paleness (3.4%). As at the time of sampling, 16 (5.0%) households indicated that there was at least one diarrhoeic animal among the animals in their households.

3.8. Detection of ESBL-producing Enterobacteriaceae from biological samples

Overall, phenotypic ESBL-producing coliform bacteria were detected in 53 (11.6%) of 457 samples collected from 320 animal-keeping households (Table 4). These include samples from Odeda (23.1%), Ijebu-Ode (4.1%), Remo North (10.3%) and Yewa-South (9.0%) LGAs. The 53 ESBL-producing isolates were obtained from different sources including the faeces of goats (20/113; 17.7%), dogs (15/108; 13.9%), sheep (4/46; 8.7%), chickens (3/101; 3.0%), ducks (2/5; 40.0%), turkey (1/9; 11.1%) and human (1/1; 100%) as well as from animal excreta within and around human residences (6/18; 33.3%) and human food (1/2; 50.0%) sources. There were no significant differences in the prevalence of ESBL-producing bacteria in goat, sheep and dogs. However, the prevalence of ESBL-producing bacteria was significantly higher ($p < 0.05$) in each of goats, sheep and dogs than in chickens. The ESBL-producing isolates were identified as *E. coli* (n = 49) and *Klebsiella pneumoniae* (n = 4). The detection of ESBL-producing bacteria in biological samples did not correlate with the hygiene score, antimicrobial usage (in humans and animals), common diseases (in humans and animals), animal access to human habitations and the presence of animal faeces in the environment of households in all the communities investigated.

3.9. ESBL genes and E. coli phylogenetic groups

In this study, the ESBL-producing isolates harboured $bla_{CTX\text{-}M\text{-}15}$ (n = 49), $bla_{CTX\text{-}M\text{-}14}$ (n = 2), $bla_{CTX\text{-}M\text{-}27}$ (n = 1) and $bla_{CTX\text{-}M\text{-}55}$ (n = 1) ESBL gene variants (Supplementary document S3). The $bla_{CTX\text{-}M\text{-}14}$ variant was detected in *E. coli* isolates from a chicken in Odeda, Odeda LGA and a duck in Ajilete, Yewa-South LGA. The $bla_{CTX\text{-}M\text{-}27}$ was detected in an *E. coli* isolate from chicken in Osielle, Odeda LGA while the $bla_{CTX\text{-}M\text{-}55}$ was detected in an *E. coli* isolate from a duck in Ijoku-Ososa, Ijebu-Ode LGA. In addition, bla_{TEM} and bla_{SHV} genes were detected in 29 and 3 isolates respectively. The bla_{TEM} gene was present in *E. coli* (n = 26) and *K. pneumoniae* (n = 3). The bla_{SHV} gene was found only in *K. pneumoniae* isolates (n = 3). In the presence of $bla_{CTX\text{-}M}$, the ESBL status of both bla_{TEM} and bla_{SHV} genes was not determined.

Forty-eight ESBL-producing *E. coli* isolates belonged to phylogenetic groups A (n = 17), B1 (n = 14), D (n = 13) and F (n = 4) while the phylogenetic group of one *E. coli* isolate could not be determined (Supplementary document S3). *E. coli* of phylogenetic group A were detected in goats (n = 5), dogs (n = 4), sheep (n = 2), chickens (n = 3), an environmental sample (n = 1), a duck (n = 1) and a turkey (n = 1). Phylogenetic group B1 isolates were from goats (n = 9), sheep (n = 2), a chicken (n = 1), an environmental sample (n = 1) and a human food sample (n = 1). Phylogenetic group D isolates were detected in goats (n = 5), dogs (n = 7), and an environmental sample (n = 1) while phylogenetic group F isolates originated from a goat (n = 1) and dogs (n = 3).

Table 4
Phenotypic detection of ESBL-producing coliforms from animals and environmental sources in households of peri-urban and rural areas of four Local Government Areas Ogun State, Nigeria.

LGA[a]	Sampling location	Sample size	Phenotypically confirmed ESBL-producing isolates (%)
Odeda	Alabata	25	15 (60.0)
	Osielle	26	5 (19.2)
	Odeda	35	6 (17.1)
	Ilugun	31	1 (3.2)
Sub-total		**117**	**27 (23.1)**
Ijebu-Ode	Ijoku-Ososa	32	2 (6.3)
	Isiwo	35	1 (2.9)
	Itemapako	26	0 (0)
	Porogun	29	2 (6.9)
Sub-total		**122**	**5 (4.1)**
Remo North	Isara	30	1 (3.3)
	Iperu-Remo	26	9 (34.6)
	Ode-Remo	26	1 (3.8)
	Ipara-Remo	25	0 (0)
Sub-total		**107**	**11 (10.3)**
Yewa-South	Ilaro	28	0 (0)
	Idogo	30	4 (13.3)
	Owode	28	4 (14.3)
	Ajilete	25	2 (8.0)
Sub-total		**111**	**10 (9.0)**
Overall total		**457**	**53 (11.6)**

[a] LGA: Local Government Area.

3.10. Antimicrobial resistance

In addition to being resistant to the β-lactam antibiotics (ampicillin, cefotaxime and ceftazidime), all ESBL-producing isolates were resistant to tetracycline. They also showed varying percentages of resistance to streptomycin (73.6%), nalidixic acid (84.9%), sulfamethoxazole/trimethoprim (81.1%), compound sulfonamides (81.1%), trimethoprim (83.0%), gentamicin (35.8%), chloramphenicol (37.7%), ciprofloxacin (28.3%), kanamycin (20.8%) and amikacin (9.4%). All the ESBL-producing isolates demonstrated multidrug resistance to at least three antimicrobial agents belonging to different classes of antimicrobials (Supplementary document S3).

4. Discussion

Animal owners in many households demonstrated low level of knowledge on antimicrobials and their usage. This could lead to problems such as wrong indication for antimicrobial, over- and under-

dosing as well as poor storage and mishandling of antimicrobial agents. Awareness about antimicrobial resistance and possible transmission of antimicrobial resistant bacteria between humans and animals was poor among household animal owners. Hence, there were no conscious efforts by household animal owners to prevent the possible transmission of microorganism between humans and animals. Human household members engaged in unorthodox healthcare practices to treat disease symptoms without taking advantage of the government healthcare facilities in the communities. Although medical centres were available in the rural and peri-urban communities investigated, people preferred self-medication and unconventional approaches (such as seeking help from drug-store owners and attendants) in the treatment of diseases. Non-prescriptional antimicrobial usage was a very common practice among humans in the communities. Antimicrobial drugs could be readily purchased from drug stores in the communities without prescription. This unrestricted access to antimicrobial agents constitute a major challenge to prudent antimicrobial usage and requires better regulations including establishment of national programme for monitoring of antimicrobial usage as well as proper enforcement of existing legislatures with appropriate penalties for offenders. Antimicrobial usage in animals was less reported than in humans. Unlike in human medicine, access to veterinary services and veterinary drug stores was limited in the communities. Hence, animal owners administered human antimicrobial preparations to their animals. Regrettably, in many occasions, sick animals were culled by slaughter for household or public consumption. Slaughtering sick animals for human consumption after initial treatment with antimicrobials (without observation of the withdrawal periods) could lead to the consumption of antimicrobial residues deposited in animal tissues by humans and exposure of the gut microflora to sub-therapeutic doses of antimicrobials. This could lead to health hazards including development of antimicrobial resistance in the exposed microflora and allergic reactions.

Phenotypic ESBL-production was detected in bacteria from animals, environmental and food sources within households of rural and peri-urban communities. The prevalence of ESBL-producing *E. coli* varied among the animal species. This could be due to the divergence in sample sizes from these species. However, findings from this study suggest that goats, sheep and dogs are more important as carriers of ESBL-producing *E. coli* than chickens within the study area. An earlier study has reported low detection rate of ESBL-producing *E. coli* in chickens from Ogun State, Nigeria (Ojo et al., 2016b). Thus, it will be instructive to pay more attention to goats, dogs and sheep as vehicles for zoonotic transmission of ESBL-producing bacteria in rural and peri-urban household settings. Any preventive strategy for the prevention of household zoonotic transmission and environmental dissemination of ESBL-producing bacteria in rural and peri-urban communities can target these three species. Unfortunately, samples could not be collected from humans because of cultural practices and superstitious beliefs of the people. However, an ESBL-producing *K. pneumoniae* that harboured $bla_{\text{CTX-M-15}}$ was isolated from faecal sample of an apparently healthy child in a household. The detection of ESBL-producing bacteria was highest in animals from Odeda LGA. Thus, people from Odeda LGA are likely to be more exposed to the risk of acquiring ESBL-producing bacteria from household animals than people from other LGAs investigated in this study. The detection of ESBL-producing bacteria was lowest in Ijebu-Ode LGA implying a comparative lower risk of household exposure to ESBL-producing bacteria of animal origins among people from Ijebu-Ode LGA than in people from other three LGAs. Studies on the molecular basis of ESBL-production in bacteria from Nigerian animal populations are scarce (Olowe et al., 2015; Ojo et al., 2016b). Most of the earlier studies only reported the phenotypic detection of ESBL-producing bacteria in samples of animal origins without investigating the genetic determinants of the ESBL traits (Chah and Oboegbulem, 2007; Ugwu et al., 2015). The present study is the first comprehensive study detailing the ESBL gene variants in caprine, ovine, avian and canine species as well as in food, environmental and human samples in Nigeria. The $bla_{\text{CTX-M-15}}$ variant was the most commonly encountered being present in all sample categories and in the two enteric bacterial species (*E. coli* and *K. pneumoniae*) across the 12 different geographical locations in Ogun State, Nigeria. Other ESBL-gene variants detected in this study were $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-27}}$ belonging to the $bla_{\text{CTX-M}}$ group 9 and $bla_{\text{CTXM-55}}$ in the $bla_{\text{CTX-M}}$ group 1. ESBL-producing Enterobacteriaceae harbouring the $bla_{\text{CTXM-15}}$ gene have been recovered from clinical samples from humans in different parts of Nigeria (Soge et al., 2006; Iroha et al., 2011, 2012; Aibinu et al., 2012; Raji et al., 2015) but the non-$bla_{\text{CTXM-15}}$ ESBL variants have not been previously reported in Nigeria. Globally, the $bla_{\text{CTXM-15}}$ and $bla_{\text{CTXM-14}}$ are among the most frequently reported ESBL-gene variants and have been reported in samples of human and animal origins (Carattoli, 2008). The $bla_{\text{CTXM-15}}$ tends to be more predominant in humans than in animals (Carattoli, 2008; Valentin et al., 2014). Other studies have identified $bla_{\text{CTXM-14}}$, $bla_{\text{CTXM-55}}$, and $bla_{\text{CTXM-27}}$ in samples from animals (Carattoli, 2008; Sun et al., 2010).

Phylogenetic classification is very useful in determining the ecological niche, lifestyle and pathogenic potentials of *E. coli* isolates (Clermont et al., 2015). In this study, the most frequently encountered phylogenetic groups were A (35.4%) and B1 (29.2%). Most *E. coli* isolates belonging to phylogroups A and B1 exist as commensals (Smith et al., 2007). Nevertheless, intestinal pathogenic *E. coli* may belong to phylogroups A and B1 (Mellata, 2013). A considerable proportion of ESBL-producing *E. coli* from this study belonged to phylogroup D (27.1%). *Escherichia coli* phylogroups B2 (F) and D are the most frequently associated with clinical infections in humans (Bingen et al., 1998; Mellata, 2013; Smati et al., 2013). Only ESBL-producing *E. coli* isolates from goats and dogs were assigned to phylogroups D and F. These two animal species were among the four most commonly reared animals species in investigated households and had relatively high prevalence of ESBL-producing bacteria compared to all the animal species investigated. Therefore, the presence of potentially human pathogenic *E. coli* phylogroups in these two species could be a reflection of their level of contact with human household members and their importance in the dissemination of pathogenic ESBL-producing *E. coli* in rural and peri-urban communities.

The ESBL-producing isolates showed high degrees of resistance to tested antimicrobials similar to the reports of earlier workers in non-ESBL producing *E. coli* from large scale livestock operations and subsistence household animals sources within the study area (Ogunleye et al., 2008; Amosun et al., 2012; Ojo et al., 2012; 2014). All the isolates were multidrug resistant with resistance to more than three antimicrobials of different classes (Schwarz et al., 2010). Indiscriminate use of antimicrobial agents as observed in the present study may create selective pressure for the emergence of multidrug resistant bacteria strains. In Nigeria, indiscriminate use of antimicrobial is common in both commercial and subsistence animal production systems (Adesokan et al., 2015; Adebowale et al., 2016; Ojo et al., 2017).

The present study focused on $bla_{\text{CTX-M}}$ without further investigating bla_{TEM} and bla_{SHV}. Hence, the ESBL status of the bla_{TEM} and bla_{SHV} genes was not determined. The $bla_{\text{CTX-M}}$ ESBL is more widely distributed across different hosts range and geographical regions than bla_{TEM} and bla_{SHV} (Carattoli, 2008). The use of cefotaxime as the sole selective agent in screening samples for the phenotypic detection of ESBL-producing bacteria introduced a form of limitation to this study. Screening of samples on cefotaxime selective medium without corresponding use of ceftazidime in the screening process may favour the detection of $bla_{\text{CTX-M}}$ type ESBL-producing strains over non-$bla_{\text{CTX-M}}$ types such as bla_{TEM} and bla_{SHV}. In the absence of $bla_{\text{CTX-M}}$, cefotaxime is limited in selecting for isolates that possessed bla_{TEM} and bla_{SHV} ESBL variants. CTX-M enzymes hydrolyse cefotaxime more readily while TEM and SHV enzymes have higher affinity for ceftazidime (Bush and Jacoby, 2010).

In conclusion, this study showed that the degree of household antimicrobial usage as reported by farmers was higher in humans than in

animals probably because the people had more access to human drugs than veterinary drugs. Free-roaming animals contributed to the dispersal of multidrug resistant ESBL-producing bacteria in the community through faecal shedding and environmental contamination. Close contact between humans and animals, poor household waste disposal methods, poor environmental sanitation and other related factors could facilitate the exchange of antimicrobial resistant bacteria between human and animal household members. Findings from the present study showed that obtaining information on animal health from veterinary personnel significantly improved the household hygiene score emphasizing the importance of veterinary personnel as veritable source of information that may be useful in promoting hygienic practices towards prevention of zoonoses in rural and peri-urban animal-keeping households. Improved access to veterinary service could lead to improved household hygiene and reduced the risk of zoonotic exchange of bacteria between animals and humans through unhygienic practices. Therefore, veterinary services should be promoted and made accessible to rural and peri-urban animal owners. There should be establishment of national antimicrobial monitoring programme to document and assess the marketing and consumption of antimicrobial agents including quantity and reasons for antimicrobial usage in humans and animals. Moreover, there should be adequate enforcement of existing antimicrobial legislatures to ensure strict compliance.

Conflict of interests

None to declare

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Appendix A. Supplementary data

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