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https://dx.doi.org/10.4314/nvj.v42i1.3 **ORIGINAL ARTICLE**

Point Prevalence and Antibiogram of Cefotaxime-Resistant Enterobacteriaceae Isolated from Food Animals and In-Contact Humans at Abattoirs, Animal Market, and Farms in Southeast, Nigeria

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SUMMARY

The spread of cefotaxime-resistant (CTX-R) Enterobacteriaceae in food animals constitutes a serious public health risk especially to humans in close proximity. We determined the point prevalence and antimicrobial resistance profile of CTX-R Enterobacteriaceae among food animals and in-contact humans in Southeast Nigeria. A total of 1,440 animals (cattle – 416, chicken – 296, goat – 287, pig – 283, sheep – 158) faecal and 406 human hand swab samples were randomly collected, enriched in peptone broth overnight and the Enterobacteriaceae were isolated on cefotaxime (5µg/ml) supplemented MacConkey agar. Antimicrobial susceptibility testing (AST) was performed on 430 randomly selected isolates from Abia (124), Ebonyi (170), and Enugu (145). The overall point prevalence of CTX-R Enterobacteriaceae in Southeast Nigeria was 84% (1549/1846) while the rates ranged between 76 ± 2.0% (95% CI 72 – 80%) to 88 ± 1.8 % (95% CI 84 – 91%) among the five states. All the 430 isolates subjected to AST were resistant to cefotaxime, ampicillin, and aztreonam while 91%, 80%, 76%, and 66% were resistant to ceftazidime, sulfamethoxazole /trimethoprim, tetracycline, and streptomycin respectively. Less than 40% of the isolates were resistant to amoxicillin/clavulanic acid, ciprofloxacin, gentamicin, norfloxacin, enrofloxacin, meropenem, and ofloxacin. The predominant resistance pattern in this study was ceftazidime-cefotaxime-aztreonam-ampicillin-streptomycin sulfamethoxazole/trimethoprim-tetracycline. The resistances observed among the isolates varied between abattoir, animal market and farms isolates. However, over 96% of the isolates had a multiple antibiotic resistance index (MARI) greater than 0.2, indicating a high-risk source of contamination.

Keywords: Antimicrobial Resistance, Enterobacteriaceae, Cefotaxime-resistance, Southeast Nigeria

INTRODUCTION

Resistance to third-generation cephalosporins (e.g. cefotaxime and ceftazidime) by members of the family *Enterobacteriaceae* has been identified as a critical emerging issue of public concern by the World Health Organization (WHO), and these resistant bacteria have been classified as high priority pathogens (Adegoke *et al.,* 2020; WHO, 2017). Among the *Enterobacteriaceae,* commensal *Escherichia coli* has been used as an indicator organism in monitoring antimicrobial resistance spread in food-producing animals and food (Aerts *et al.,* 2019). *Enterobacteriaceae* can become resistant to third-generation cephalosporins by the production of extended-spectrum β-lactamase (ESBL) enzymes (Founou *et al.,* 2019). This confers the ability to hydrolyze β-lactam antibiotics such as penicillins, cephalosporins, and monobactam, but not cephamycin and carbapenem (Abrar *et al.,* 2018). Cefotaxime is classified as one of the highest priority critically important antimicrobials (HP-CIA) for human use and there is evidence in the scientific literature that its use in animals poses a public health risk to humans as a result of the development of AMR, which may be transferred through the food chain (Medicines Directorate, 2017). Resistance to cefotaxime (an oxyimino-β-lactam) was first described in 1980 and since then, there has been an exponential increase in its global incidence and spread (Faari *et al.,* 2015). ESBL producing *Enterobacteriaceae* was originally a concern in the human clinical setting as they were identified in nosocomial *Klebsiella pneumoniae* and *Escherichia coli* outbreaks (Pérez *et al.,* 2019). Recently, there have been reports of increasing incidence of ESBL-producing *Enterobacteriaceae* in animal populations that may not have been treated with cephalosporins (Markland *et al.,* 2019; Velasova *et al.,* 2019). Globally, intensive efforts have been invested in AMR monitoring reflected by increased knowledge about the cause, consequences and, prevention of AMR, as well as the development and implementation of AMR action plans by several countries (O'Neill, 2014; Wernli *et al.,* 2017). However, the burden of AMR may be underestimated in Nigeria, where infectious diseases still account for 63% of preventable premature mortalities (Ebimieowei & Ibemologi, 2016; Neill, 2015; WHO, 2018). Furthermore, the gap in knowledge about AMR in Nigeria may be due to the inadequate established structures to collate holistic epidemiological data from rural and suburban regions. There is currently a paucity of information on the prevalence of cefotaxime resistant (CTX-R) *Enterobacteriaceae* in Nigeria. The available point prevalence (prevalence measured at a particular point in time) has been limited to reports from human tertiary hospitals (Nwafia *et al.,* 2019; Oli *et al.,* 2019; Olowe *et al.,* 2015; Olowe *et al.,* 2013; Olowo-okere *et al.,* 2018). The few reports available from Southeast, Nigeria, indicated that the point prevalence in humans and animals ranged from 2.5% to 76.9% (Iroha *et al.,* 2012) and 9.4% to 25.4% (Chah *et al.,* 2010), respectively. Therefore, the objectives of this study were to determine the prevalence of CTX-R *Enterobacteriaceae* in randomly selected healthy livestock and in-contact humans in Southeast, Nigeria, and to determine the antimicrobial susceptibility profile of the isolates from different host species.

MATERIALS AND METHODS

Ethical statement

The methods/procedures used in this study were concomitant with that outlined in the Animals Scientific Procedures Act of 1986 for the care and use of animals for research purposes. Approval was obtained from the Research Ethics Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (Approval Reference Number: FVM-UNN-IACUC-2019-0570).

Study area

This study was conducted in the Southeast geopolitical zone of Nigeria, which comprised five states namely: Abia, Anambra, Ebonyi, Enugu, and Imo States. Southeast Nigeria, also known as Igbo land, is the indigenous homeland of the Igbo people. Geographically, Anambra, Ebonyi, Enugu are bordered by northern states while Imo and Abia are bordered by southern states. More than 30million people inhabit Igbo land with a population density ranging from 140 to 390 inhabitants per square kilometre (350/1,000sq mi). Altogether Igbo land has an area of 40,900 to 41,400km^2 (Nigerian Finder, 2019).

Sampling and Isolation of bacteria

A two-stage cluster sampling was employed. In the first stage cluster sampling, a total of 10, 12, and 50 abattoirs, animal markets, and farms were selected respectively from the five Southeast states based on the population of food animals (> 50 animals) at each sampling site. In the second stage, systematic random sampling was employed to randomly collect faecal swabs from one out of every 10 healthy livestock (a maximum of 10 faecal rectal swabs were collected from cattle, chicken, goat, pig, and sheep per time) and non-probability convenience sampling was employed to take hand swabs from willing in-contact humans at each sampling site. The faecal rectal (n = 1,440) and hand swab (n = 406) samples were first enriched in peptone broth overnight before a loopful of the enriched samples were plated on cefotaxime (5µ/ml) supplemented MacConkey agar and incubated at 37^{0}C for 18 - 24 h. Positive samples were identified as plates with more than 10 colonies. One non-duplicate lactose fermenting colony was selected from each primary plate and purified by sub-culturing. The purified colonies were subjected to biochemical identification using the API20E kit (bioMerieux, SA, Marcy-l'Étoile, France).

Antimicrobial Susceptibility Testing of Selected CTX-R Enterobacteriaceae

Four hundred and thirty representative CTX-R *Enterobacteriaceae* isolates comprising *Escherichia coli* (73%), *Klebsiella pneumoniae* (19%), and *Enterobacter* spp (8%) were selected by simple random sampling technique for antimicrobial susceptibility testing (AST) with 14 antimicrobials. The disc diffusion method and the resistance breakpoint were determined by Clinical and Laboratory Standards Institute guidelines (CLSI, 2017). Post incubation, the susceptibility of the isolates to each antimicrobial agent was evaluated by measuring the inhibition zone diameter (IZD) around each disc using a digital Vernier calliper (Mitutoyo ABSOLUTE AOS) and was recorded in millimetres. Each isolate was classified as either sensitive, intermediate, or resistant.

Multiple Antibiotic Resistance Index

The multiple antimicrobial resistance (MAR) index of each cefotaxime resistant *Enterobacteriaceae* subjected to antimicrobial susceptibility test was calculated using the formula MAR = a/b where 'a' is the total number of antibiotics the isolate was resistant to and 'b' is the total number of antibiotics the test isolate was subjected to for susceptibility. Isolates with MAR index values greater than 0.2 were considered to originate from a high-risk source of contamination where several antibiotics are often used (Sandhu, 2016).

Data presentation and analysis

The descriptive data generated were analysed using IBM SPSS version 25 (New York, US). The prevalence of cefotaxime resistant *Enterobacteriaceae* in the five Southeast states in Nigeria was visualized using a choropleth map designed with ArcGIS software v 3.4 (Aylesbury, UK) and the associations between the presence of CTX-R *Enterobacteriaceae* and state of origin of samples, sampling levels, and animal species were analysed using Pearson Chi-square. The antimicrobial resistance profile of the 430 non-clinical isolates from food animals and in-contact humans were subjected to dimensional-reduction using principal component analysis (PCA) to visualize the variations in the large data and explain the maximal amount of variance that exists between the 14 antimicrobials tested in this study.

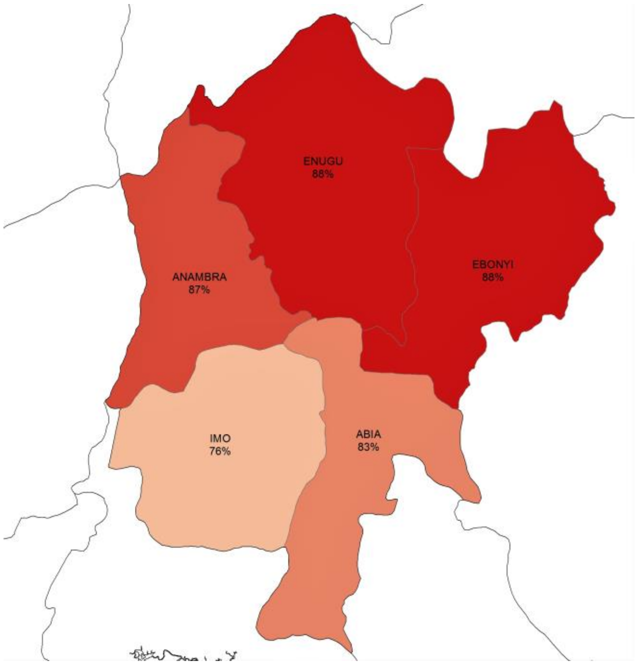

Figure 1. Choropleth map of the prevalence of CTX-R *Enterobacteriaceae* in Southeast, Nigeria. Ebonyi, Enugu, and Anambra are bordered by Northern states while Imo and Abia are bordered by Southern states

RESULTS

The overall point prevalence of CTX-R *Enterobacteriaceae* in Southeast, Nigeria was 84% (1549/1846) and ranged from 76 ± 2.0 (95% CI 72 – 80%) to 88 ± 1.8 % (95% CI 84 – 91%) between states (Table I).

TABLE I. Point prevalence of cefotaxime resistant *Enterobacteriaceae* in Southeast, Nigeria

	Total number of samples processed (N)	**Percentage Resistant ± SE* (%)**	**95% Confidence Interval**	
			Lower bound (%)	**Upper bound (%)**
Location				
Abia	352	83 ± 2.0	79	83
Anambra	428	88 ± 1.6	84	91
Ebonyi	301	87 ± 1.9	83	91
Enugu	322	88 ± 1.8	84	91
Imo	443	76 ± 2.0	72	80
Sample origin				
Abattoir	665	86 ± 1.3	84	89
Animal market	369	82 ± 2.0	78	86
Farm	812	83 ± 1.3	80	85
Species				
Cattle	416	84 ± 1.8	80	87
Chicken	296	76 ± 2.5	71	81
Goat	287	80 ± 2.4	75	85
Human	406	88 ± 1.6	85	91
Pig	283	83 ± 2.2	79	88
Sheep	158	96 ± 1.5	93	99

***95% confidence interval**

The spatial distribution of the prevalence of CTX-R *Enterobacteriaceae* between the states as visualized on a choropleth map showed that all the Southeast states sharing a boundary with northern states (Anambra, Ebonyi, and Enugu) recorded higher point prevalence of CTX-R *Enterobacteriaceae* than those bordered by southern states -Imo and Abia (Figure 1). There was a significant association (X^2(4, N = 1846) = 30.715, p <0.001) between the occurence of CTX-R *Enterobacteriaceae* and the different states in Southeast, Nigeria. Among the three sampling levels, abattoir isolates recorded the highest prevalence estimates of CTX-R *Enterobacteriaceae* (Table I). The prevalence estimates were 86% (95% CI 84 - 89%), 82% (95% CI 78 - 86%) and 83% (95% CI 80 - 85%) from samples collected from abattoirs, animal markets, and farms respectively. There was no significant association (X^2(2) = 4.53, p =0.104) between the occurence of CTX-R *Enterobacteriaceae* and the different sample levels (abattoir, animal market, and farms). The prevalence estimates for CTX-R *Enterobacteriaceae* in different livestock species was between 76% (95% CI 71 - 81%) and 96% (95% CI 93 - 99%), and was 88% (95% CI 85 – 91%) in humans (Table I). Samples obtained from chicken recorded the lowest prevalence and those from sheep recorded the highest prevalence estimates. Pearson's Chi-Squared test revealed a significant association (X^2(5) = 38.173, p <0.001) between the presence of CTX-R *Enterobacteriaceae* and the different host species sampled.The 430 *Enterobacteriaceae* isolates (*Escherichia coli* – 73%, *Klebsiella pneumonia* – 19%, and *Enterobacter cloacae* - 8%) subjected to AST were all resistant to cefotaxime, aztreonam, and ampicillin while 91%, 80%, 76%, and 66% were resistant to ceftazidime, sulfamethoxazole /trimethoprim, tetracycline, and streptomycin, respectively (Table II). Less than 40% of the isolates were resistant to amoxicillin/clavulanic acid, ciprofloxacin, gentamicin, norfloxacin, enrofloxacin, meropenem, and ofloxacin. In addition to the 100% resistance to cefotaxime, aztreonam, and ampicillin, a greater proportion of the isolates from abattoir samples were observed to be resistant to meropenem (40%, 52/131), amoxicillin / clavulanic acid (50%, 66/131), gentamicin (35%, 46/131), and streptomycin (74%, 97/131) while a greater proportion of those isolated from the animal markets was resistant to the fluoroquinolones and sulfamethoxazole /trimethoprim (Table II).

TABLE II. Antibiogram of cefotaxime resistant *Enterobacteriaceae* from food animals and in-contact humans in the South East, Nigeria (n=430)

Class of Antimicrobial	Antimicrobial	Resistance break point* (mm)	Overall resistant isolate n=430 (%)	Abattoir n= 131 (%)	Animal market n=93 (%)	Farms n=206 (%)
Carbapenem	Meropenem (10µg)	**≤ 19**	117 (27)	52 (40)	16 (17)	49 (24)
Third generation cephalosporin	Ceftazidime (30µg)	**≤ 17**	392 (91)	120 (92)	83 (89)	189 (92)
	Cefotaxime (30µg)	**≤ 22**	430 (100)	131 (100)	93 (100)	206 (100)
Penicillin	Ampicillin (10µg)	**≤ 17**	430 (100)	131 (100)	93 (100)	206 (100)
Monobactam	Aztreonam (30µg)	**≤ 13**	430 (100)	131 (100)	93 (100)	206 (100)
β-Lactam inhibitor	Amoxycillin/ clavulanic acid (30µg)	**≤ 13**	164 (38)	66 (50)	30 (32)	68 (33)
Quinolone and fluoroquinolone	Ofloxacin(5µg)	**≤ 12**	111 (26)	33 (25)	28 (30)	50 (24)
	Norfloxacin (10µg)	**≤ 12**	120 (28)	36 (27)	28 (30)	56 (24)
	Ciprofloxacin (10µg)	**≤ 15**	149 (35)	47 (36)	38 (41)	64 (31)
	Enrofloxacin(5µg)	**≤ 12**	118 (27)	36 (27)	31 (33)	51 (25)
Aminoglycoside	Gentamicin (10µg)	**≤ 12**	135 (31)	46 (35)	22 (24)	67 (33)
	Streptomycin(5µg)	**≤ 11**	285 (66)	97 (74)	66 (71)	122 (59)
Folate pathway inhibitor	Sulfamethoxazole/ trimethoprim (25µg)	**≤ 10**	344 (80)	102 (78)	77 (83)	165 (80)
Tetracycline	Tetracycline (30µg)	**≤ 11**	328 (76)	102 (78)	66 (71)	160 (78)

*****(CLSI., 2017)

Isolates from abattoir and farm samples were equally resistant to ceftazidime (92%, 120/131 and 92%, 189/206) and tetracycline (78%, 102/131, and 160/206) and were higher than the percentage of isolates from animal market samples resistant to the same antimicrobials.

TABLE III. Recurrent antimicrobial resistance pattern of Enterobacteriaceae

Patterns	**Frequency**
CAZ+CTX+ATM+AMP+S+STX+TE	38 (8.7%)
CAZ+CTX+ATM+AMP+CN+S+STX+TE	27 (6.2%)
CAZ+CTX+ATM+AMP+OFX+NOR+CIP+ENR+S+STX+TE	23 (5.2%)
CAZ+CTX+ATM+AMP+CN+STX+TE	21 (4.8%)
MEM+CAZ+CTX+ATM+AMP+AMC+S+STX+TE	21 (4.8%)
CAZ+CTX+ATM+AMP	17 (4.0%)
CAZ+CTX+ATM+AMP+AMC+OFX+NOR+CIP+ENR+STX+TE	13 (3.2%)
CTX+ATM+AMP+S+STX+TE	11 (2.6%)
CAZ+CTX+ATM+AMP+AMC+OFX+NOR+CIP+ENR+S+STX+TE	11 (2.6%)
CAZ+CTX+ATM+AMP+AMC+S+STX+TE	10 (2.3%)
CAZ+CTX+ATM+AMP+AMC+OFX+NOR+CIP+ENR+CN+S+STX+TE	10 (2.3%)
MEM+CAZ+CTX+ATM+AMP	10 (2.3%)
CAZ+CTX+ATM+AMP+OFX+NOR+CIP+ENR+STX+TE	9 (2.1%)
CAZ+CTX+ATM+AMP+OFX+NOR+CIP+ENR+CN+S+STX+TE	9 (2.1%)
MEM+CAZ+CTX+ATM+AMP+AMC	9 (2.1%)
MEM+CAZ+CTX+ATM+AMP+AMC+OFX+NOR+CIP+ENR+CN+S+STX+TE	9 (2.1%)
CAZ+CTX+ATM+AMP+CIP+S+STX+TE	7 (1.6%)
CTX+ATM+AMP	6 (1.4%)
MEM+CAZ+CTX+ATM+AMP+STX+TE	6 (1.4%)
MEM+CAZ+CTX+ATM+AMP+AMC+CN+S+STX+TE	6 (1.4%)
CAZ+CTX+ATM+AMP+TE	5 (1.2%)
CAZ+CTX+ATM+AMP+S	5 (1.2%)
CAZ+CTX+ATM+AMP+CIP+CN+S+STX+TE	5 (1.1%)
CAZ+CTX+ATM+AMP+AMC+CN+S+STX+TE	5 (1.1%)
CAZ+CTX+ATM+AMP+STX+TE	4 (0.9%)
CAZ+CTX+ATM+AMP+S+STX	4 (0.9%)
CAZ+CTX+ATM+AMP+CN+S+STX	4 (0.9%)
CAZ+CTX+ATM+AMP+AMC+STX+TE	4 (0.9%)
MEM+CAZ+CTX+ATM+AMP	4 (0.9%)
MEM+CAZ+CTX+ATM+AMP+STX+TE	4 (0.9%)
MEM+CAZ+CTX+ATM+AMP+CIP+S+STX+TE	4 (0.9%)
MEM+CAZ+CTX+ATM+AMP+AMC+S	4 (0.9%)

MEM- Meropenem, CAZ-Ceftazidime, CTX- Cefotaxime, ATM- Aztreonam, AMP- Ampicillin, AMC- Amoxicillin/clavulanic acid (Amoxiclav), OFX- Ofloxacin, NOR- Norfloxacin, CIP-Ciprofloxacin, ENR- Enrofloxacin, CN- Gentamicin, S- Streptomycin, STX-Sulfamethoxazole/trimethoprim, TE- Tetracycline

TABLE IV. Predominant phenotypic AMR pattern at different sampling levels

Sample Origin	Predominant Phenotypic AMR Pattern
State	
Abia	CAZ, CTX, ATM, AMP, S, STX, TE
Ebonyi	CAZ, CTX, ATM, AMP, OFX, NOR, CIP, ENR, S, STX, TE
Enugu	CAZ, CTX, ATM, AMP, S, STX, TE
Sampling Level	
Abattoir	MEM, CAZ, CTX, ATM, AMP, AMC, S, STX, TE
Animal Market	CAZ, CTX, ATM, AMP, S, STX, TE
Farms	CAZ, CTX, ATM, AMP, S, STX, TE
Species	
Cattle	MEM, CAZ, CTX, ATM, AMP, AMC, S, STX, TE
Chicken	CAZ, CTX, ATM, AMP, CN, STX, TE
Goat	CAZ, CTX, ATM, AMP, S, STX, TE
Human	CAZ, CTX, ATM, AMP, CN, S, STX, TE
Pig	CAZ, CTX, ATM, AMP, S, STX, TE
Sheep	CAZ, CTX, ATM, AMP, OFX, NOR, CIP, ENR, S, STX, TE

MEM- Meropenem, CAZ-Ceftazidime, CTX- Cefotaxime, ATM- Aztreonam, AMP-Ampicillin, AMC- Amoxicillin/clavulanic acid (Amoxiclav), OFX- Ofloxacin, NOR-Norfloxacin, CIP- Ciprofloxacin, ENR- Enrofloxacin, CN- Gentamicin, S- Streptomycin, STX-Sulfamethoxazole/trimethoprim, TE- Tetracycline

A total of 106 different antimicrobial resistance patterns were observed in the 430 Enterobacteria screened. Seventy-six different patterns were present in 3 or fewer isolates and are not included in Table III. The predominant phenotypic AMR pattern identified among all the isolates from food animals and in-contact humans in Southeast, Nigeria included resistance to ceftazidime, cefotaxime, aztreonam, ampicillin, streptomycin, sulfamethoxazole/trimethoprim, and tetracycline. Isolates from different sampling levels had varying reoccurring phenotypic AMR patterns Table IV. Isolates obtained from Abia, and Enugu had the same predominant phenotypic AMR patterns but those obtained from Ebonyi had in addition to the predominant phenotypic AMR pattern, resistance to all the fluoroquinolones used in the study. The most common phenotypic AMR pattern among abattoir isolates included resistance to meropenem, ceftazidime, cefotaxime, aztreonam, ampicillin, amoxicillin/clavulanic acid, streptomycin, sulfamethoxazole/trimethoprim, and tetracycline. In contrast, the most frequently detected phenotypic AMR pattern in isolates from the animal markets and farms included resistance to ceftazidime, cefotaxime, aztreonam, ampicillin, streptomycin, sulfamethoxazole/trimethoprim, and tetracycline. Isolates obtained from different species had varying predominant phenotypic AMR patterns. Isolates from goats and pigs had the same predominant phenotypic AMR pattern while those from chicken had resistance to gentamicin in addition to the pattern observed in goat and pig isolates. Similarly, human isolates were observed to also have gentamicin and streptomycin resistance phenotypes in addition to the predominant AMR phenotypes observed in goat and pig isolates. The predominant phenotypic AMR pattern among cattle isolates included resistance to meropenem, ceftazidime, cefotaxime, aztreonam, ampicillin, amoxicillin/ clavulanic acid, streptomycin, sulfamethoxazole/trimethoprim, and tetracycline. The PCA showed four-component clustering of the inhibition zone diameter of the isolates. It was observed that all the four antimicrobials belonging to the fluoroquinolone class (Ofloxacin, enrofloxacin, ciprofloxacin, and norfloxacin) used in the AST clustered together in the same quadrant while the second, third, and fourth clusters had different classes of antimicrobials clustering together in the same quadrant (Figure 2). The most frequently detected antimicrobial resistance pattern in the isolates from the three states predominantly (38/430, 8.7%) include ceftazidime-cefotaxime-aztreonam-ampicillin-streptomycin-sulfamethoxazole/trimethoprim-tetracycline resistances. The MAR index (Figure 3) revealed 416 isolates (96.7%) with MAR index greater than 0.2 Although 105 isolates (24.4%) had the MAR index of 0.6, nine isolates were observed to have an index of 1.0 as they showed resistance to all the 14 antimicrobials they were subjected to. The resistant *Enterobacteriaceae* isolates with MAR of greater than 0.2 were distributed across all the sampling levels (abattoir, animal market, and farms).

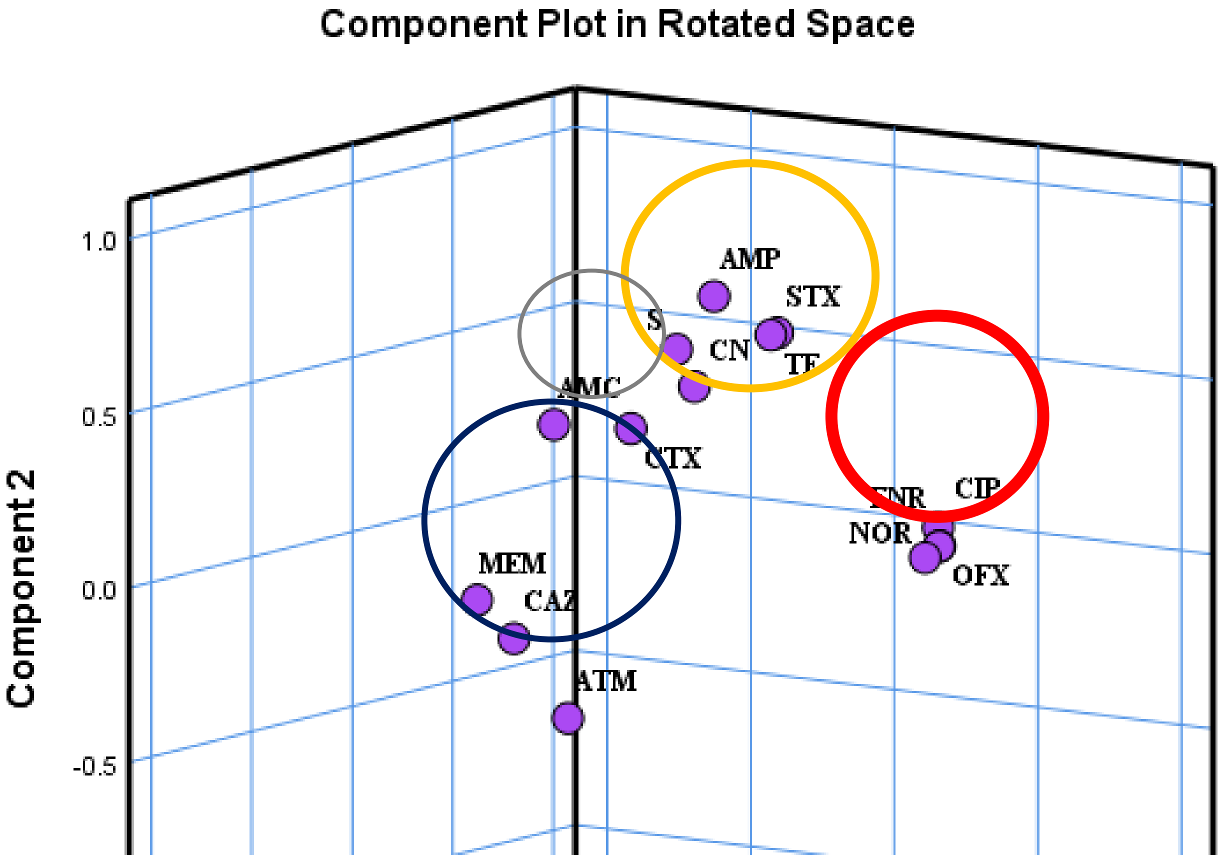

Figure 2 Principal component analysis showing the multi-drug resistant patterns of *Enterobacteriaceae* isolated from food animals and in-contact humans in southeast Nigeria
Meropenem (MEM), Ceftazidime (CAZ), Cefotaxime (CTX), Aztreonam (ATM), Ampicillin (AMP), Amoxicillin/clavulanic acid (AMC), Enrofloxacin (ENR), Ofloxacin (OFX), Norfloxacin (NOR), Ciprofloxacin (CIP), Gentamicin (CN), Streptomycin (S), sulphamethoxazole/trimethoprim (STX) and Tetracycline (TE)

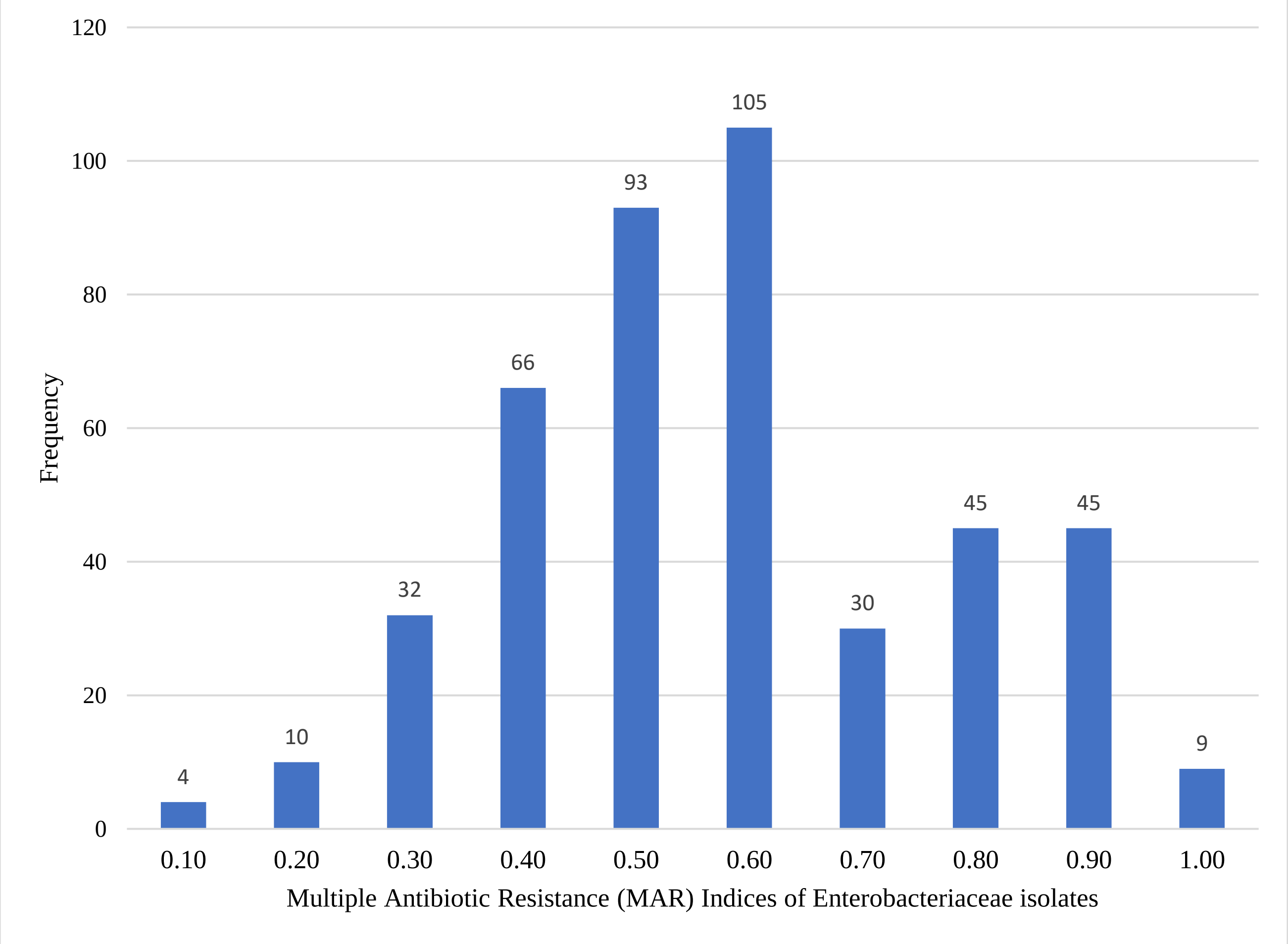

Figure 3 Multiple antibiotic resistance (MAR) indices for *Enterobacteriaceae* from Food animals and in-contact humans in southeast Nigeria

DISCUSSION

To the best of the knowledge of the authors, this is the first comprehensive study on the prevalence of CTX-R *Enterobacteriaceae* in Southeast, Nigeria. Hence, to have a broad understanding of the prevalence of CTX -R *Enterobacteriaceae* in Southeast Nigeria, samples were taken from food animals and humans at the points where there is a close interface between humans and food animals (abattoir, animal markets, and farm). The prevalence of CTX-R *Enterobacteriaceae* in the Southeast geopolitical zone of Nigeria was found to be 84% from our findings and this result is within the upper range (0 -87%) of resistance to third-generation cephalosporins (3GCs) published by the WHO Antimicrobial Resistance Global Report on Surveillance gathered from all data including publications available from Africa (WHO, 2014). Although there were relatively minor variations in the prevalence of CTX-R *Enterobacteriaceae* obtained in each of the five states, states that serve as a gateway to the southeast (Anambra, Enugu, and Ebonyi) and bordered by northern states were observed to have higher prevalence rates than those located southwards (Imo and Abia) and bordered by the Southern States. The larger proportion of the Nigerian livestock is concentrated in the northern states in the care of pastoralists and it has been estimated that Nigeria has a population of 34.5 million goats, 22.1 million sheep, and 13.9 million cattle (Lawal-Adebowale, 2012). It has been reported that most pastoralists find the veterinary services too expensive and so resort to indiscriminate use of antimicrobials for treatment and prevention of diseases especially during the course of on-hoof migration down south in search for pasture during the time of drought (usually between October and May) (Alhaji and Isola, 2018; NCDC, 2017).

Cefotaxime is one of the best drugs of choice in treating several high-priority infections in humans, and thus resistance to this antibiotic in the human population could be explained by the overuse and misuse of antibiotics (Faari *et al.,* 2015; Markland *et al.,* 2019). However, to the best of our knowledge, cefotaxime is not used in food animals in Southeast Nigeria, hence, the high prevalence of CTX-R *Enterobacteriaceae* isolates among food animals (76 to 96%) observed in this study presents a serious public health concern. *Enterobacteriaceae* may acquire resistance genes from antibiotic-producing microorganisms in the environment thereby potentiating the evolution and spread of natural antibiotics resistomes (Mir *et al.,* 2016). The observation in this study that over 80% of the isolates were resistant to semi-synthetic antibiotics (cefotaxime) especially in food animals with no obvious previous exposure is worrisome. There have been several reports of CTX-R *Enterobacteriaceae* in animals not exposed to 3GCs (Hille *et al.,* 2017; Markland *et al.,* 2019; Mir *et al.,* 2016) especially in cattle. The prevalence of CTX-R *Enterobacteriaceae* isolates from chicken and pigs were lower than those isolated from cattle, humans, goat, and sheep. Although poultry and piggery are the predominant farm animal ventures in the South East, they are mostly backyard or small-scale ventures that are not sufficient to meet the daily animal protein requirements of the populace in the region (Nwanta *et al.,* 2011). These animals are confined in houses and are raised for a short period before culling as compared with ruminants that are mostly raised for several years and exposed to more antimicrobials during their lifetime. Ruminants are more frequently slaughtered at the abattoir and so it was not out of place to find the highest proportion of CTX-R *Enterobacteriaceae* in isolates obtained from the abattoir as it serves as a convergent location for animals from different regions, farms, and markets. The unhygienic sanitary condition of abattoirs in the southeast could also be a contributing factor to the spread and high prevalence of CTX-R *Enterobacteriaceae* as there are no appropriate strategies/ management for efficient abattoir solid waste disposal (Bose and Kime, 2019; Ezeoha and Ugwuishiwu, 2011; Nwanta *et al.*, 2010).

Although 44% of the total samples collected were from farms with no previous history of cefotaxime use in production, the prevalence of CTX-R *Enterobacteriaceae* was above 80%. The pathway to the dissemination and widespread resistance to cefotaxime in this scenario is not clearly understood but may be from environmental or human sources. The most common feature observed in all the farms in the southeast was poor farm waste (manure) management. The possibility of cross-contamination and re-infection is high as most farms rely on groundwater from shallow wells and boreholes as a source of drinking water for the animals. Several microbiological studies on the quality of groundwater in Nigeria have shown a high level of groundwater contamination with coliforms, *Staphylococcus aureus*, *Bacillus* spp, *Salmonella* spp, and other non-fastidious pathogens (Bello *et al.*, 2013; Bisi-Johnson *et al.*, 2017; Okoye *et al.*, 2016; Onwughara *et al.*, 2013). Horizontal transfer of resistance genes in bacteria via environmental niches such as manure, water, and soil have been reported to facilitate the spread of AMR in populations that have not been previously exposed to a particular antibiotic (Founou *et al.*, 2016).

Furthermore, the fact that ESBL producers also carry genes that confer resistance to other classes of antibiotics such as fluoroquinolones, tetracycline, aminoglycosides, and sulfonamides (Elsayed *et al.*, 2017; Mir *et al.*, 2016) may aid selection and on-farm persistence. The antimicrobial susceptibility test (AST) results of the 430 CTX-R *Enterobacteriaceae* isolated from different parts of Southeast Nigeria showed the multidrug-resistant nature of the selected isolates to various classes of antibiotics.

The use of carbapenems, 3GCs, and monobactams, without prescription, in the treatment of infections in humans, is not uncommon in Nigeria (Oduyebo *et al.*, 2015). Their use in food animals is restricted not because they are outrightly prohibited but because these drugs are expensive and usually administered parenterally, which most farmers perceive to be laborious. Resistance to these classes of antimicrobials in the food chain is worrisome and of great public health concern (Abrar *et al.*, 2018). The most commonly used antibiotics in food animal production in Nigeria are tetracyclines, aminoglycosides (especially streptomycin), and penicillins which are readily affordable and available over the counter (Alhaji and Isola, 2018; NCDC, 2017). Although there are few documented reports on the resistant profile of *Enterobacteriaceae* isolated from food animals in Nigeria, most of the published articles reported a greater proportion of *Enterobacteriaceae* isolated from food animals to be resistant to ampicillin, streptomycin, sulfamethoxazole/trimethoprim, and tetracycline (Chah *et al.*, 2018; Fashae *et al.*, 2018; Ojo *et al.*, 2016; Okpara *et al.*, 2018; Olowe *et al.*, 2015; Omoshaba *et al.*, 2017). Resistance to fluoroquinolone by the isolates in this study was less than 40% and this was similar to that reported in Southeast (Enugu) by Chah *et al.* (2018) but higher than 26.5% reported by Adesokan *et al.* (2015) in Southwest, Nigeria.

An evaluation of the resistance profile from the different sample origin revealed a significant variation between the proportion of isolates resistant to meropenem and amoxicillin/ clavulanic acid from the abattoir as compared with those from the animal market and farms. Abattoirs in southeast Nigeria are not just dedicated buildings for the slaughtering of animals and processing of the carcasses but they are also a mini-market hub that attracts individuals with unknown health status who may be carriers of resistant pathogens (Nwanta *et al.*, 2010). The high levels of resistance to the four fluoroquinolones used in this study by isolates from the animal markets as compared to those from the abattoirs and farms maybe as a result of the usual practice of drug doping using a combination of ciprofloxacin and dexamethasone/prednisolone to confer soundness to an otherwise unfit or sick animal (Thippeswamy *et al.*, 2018). Sulfamethoxazole/trimethoprim is another popular antibiotic that animal sellers administer, as they are frequently used in all cases of diarrhoea regardless of the aetiology and this may explain the high levels of resistance observed in the isolates from the animal market.

The frequent occurrence of typhoid fever caused by the consumption of contaminated food or water has propelled the high use of fluoroquinolones in humans. The proportion of isolates resistant to fluoroquinolones in this study was less than 50%. *Enterobacteriaceae* isolated from goats were observed to have a greater proportion of the isolates which showed resistance to fluoroquinolones than those from chickens, although the use of this class of drug in poultry production is common (Adesokan *et al.,* 2015; Oloso *et al.,* 2018). It was observed that an indigenous breed of goats (West African dwarf) live in proximity with humans in the Southeast, and transmission from humans may explain the proportion of resistant isolates observed in addition to drug doping commonly practiced at the animal market (Thippeswamy *et al.,* 2018). A greater proportion of the isolates from chickens were resistant to gentamicin probably because of the high usage of the drug especially during the process of brooding chicks. The available veterinary preparations of streptomycin are usually in combination with penicillins, although in the present study ~ 66% of isolates were resistant to streptomycin and this was not unusual as streptomycin is frequently used to treat most bacterial infections. Ampicillin, tetracycline and sulfamethoxazole/trimethoprim are the commonly used drugs in veterinary practice in Nigeria, thus the appearance of resistance to these drugs is not surprising but it generates a great concern for the future of livestock production in addition to the public health implication of the spread of the multidrug-resistant bacteria (Adesokan *et al.,* 2015).

The PCA provided an insight into the relationship between the multidrug resistances observed among the CTX-R *Enterobacteriaceae* from food animals and in-contact humans in Southeast Nigeria. The most striking observation in this study was the clustering of quinolone/fluoroquinolones antimicrobials indicating that CTX-R *Enterobacteriaceae* that showed resistance to one fluoroquinolone antibiotic could also be resistant to others in the same antimicrobial class. Although the mechanism of resistance to quinolone/fluoroquinolone was not queried in this study, it has been demonstrated that the development of resistance to one fluoroquinolone may confer resistance to other subtypes (Jacoby, 2005). Additionally, a cluster of four different antimicrobial classes (Penicillin, aminoglycoside, folate pathway inhibitor, and tetracycline) was observed in the PCA result which displayed the multidrug-resistant nature of CTX-R *Enterobacteriaceae* isolated from the different animal species. To the best of our knowledge, this is the first comprehensive study conducted on the prevalence of CTX-R *Enterobacteriaceae* in Southeast Nigeria, although reports from other parts of Nigeria were compiled in a review by Oloso et al. (2018), which also revealed that isolates from livestock are often multidrug-resistant (Oloso *et al.,* 2018). The common antimicrobial resistance pattern observed in this study included resistance to ceftazidime, cefotaxime, aztreonam, ampicillin, streptomycin, sulfamethoxazole/trimethoprim, and tetracycline. This finding was similar to those reported in point surveillance in the South West (Adesokan *et al.,* 2015; Okpara *et al.,* 2018). Over 96% of the isolates in this study were observed to have a MAR index greater than 0.2, indicating that the majority of the isolates originated from a high-risk source of contamination where several antibiotics are frequently used (Sandhu, 2016). ESBL-producing *Enterobacteriaceae* are known to harbour genes conferring resistance to other non-β-lactam antibiotics, hence may explain the multidrug-resistant phenotypes identified in this study.

CONCLUSION

The application of a holistic One Health approach to study the prevalence of antimicrobial-resistant isolates in food animals and in-contact humans used in this study provided an understanding of the public health implications of the distribution of bacteria that are resistant to critically important antimicrobials. It was observed that all the *Enterobacteriaceae* isolated from food animals and in-contact humans were resistant to at least one out of the two 3GCs. The gross implication of these resistances is that the use of 3GCs in this study area to treat severe infectious diseases in humans may be practically impossible and this may lead to prolonged hospitalization and death.

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