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

Olorunleke, S.O.¹; Okorie-Kanu, O.J.², Nwanta, J.A.²; Chah, K.F.³

¹Department of Animal Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.
²Department of Veterinary Public Health and Preventive Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.
³Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria.
*Corresponding author Email: solomon.olorunleke@ebsu.edu.ng; Mobile: +2348060955581

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 cephapemycin 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 (Ebimieowiei & 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-oke 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 30 million people inhabit Igbo land with a population density ranging from 140 to 390 inhabitants per square kilometre (350/1,000 sq mi). Altogether Igbo land has an area of 40,900 to 41,400 km$^2$ (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°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.
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).

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

| 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 occurrence 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 occurrence 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)       |
| Penicillin             | 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)        |
| Aminoglycoside         | Gentamicin (10µg)               | ≤ 12                         | 135 (31)                           | 46 (35)             | 22 (24)                | 67 (33)        |
| 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+S+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+CIP+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- Cefazidine, 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), sulphonmethoxazole/trimethoprim (STX) and Tetracycline (TE)

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

**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
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.

REFERENCES
ABRAR, S., HUSSAIN, S., KHAN, R. A., UL AIN, N., HAIDER, H. and RIAZ, S. (2018). Prevalence of extended-spectrum-β-lactamase-producing Enterobacteriaceae: first systematic meta-analysis report from Pakistan. Antimicrobial Resistance & Infection Control, 7(1): 26.

AEGEKE, A. A., MADU, C. E., AIYEGORO, O. A., STENSTRÖM, T. A. and OKOH, A. I. (2020). Antibiogram and beta-lactamase genes among cefotaxime resistant E. coli from wastewater treatment plant. Antimicrobial Resistance & Infection Control, 9(1): 1–12.

ADESOKAN, H. K., AKANBI, I. O., AKANBI, I. M. and OBAWEDA, R. A. (2015). Pattern of antimicrobial usage in livestock animals in South-Western Nigeria: The need for alternative plans. Onderstepoort Journal of Veterinary Research, 82(1): 6.

AERTS, M., BATTISTI, A., HENDRIKSEN, R., KEMPF, I., TEALE, C., TENHAGEN, B. A., VELDMAN, K., WASYL, D., GUERRA, B., LIEBANA, E., THOMAS-LOPEZ, D. and BELŒIL, P. A. (2019). Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food. EFSA Journal, 17(6):5709

ALHAJI, N. B. and ISOLA, T. O. (2018). Antimicrobial usage by pastoralists in food animals in North-central Nigeria: The associated socio-cultural drivers for antimicrobials misuse and public health implications. One Health, 6: 41–47.

BELLO, O. O., OSHO, A., BANKOLE, S. A. and BELLO, T. K. (2013). Bacteriological and Physicochemical Analyses of Borehole and Well Water Sources in Ijebu-Ode, Southwestern Nigeria. IOSR Journal of Pharmacy and Biological Sciences, 8(2):18-25.

BISI-JOHNSON, M. A., ADEDIRAN, K. O., AKNOLA, S. A., POPOOLA, E. O. and OKOH, A. I. (2017). Comparative physicochemical and microbiological qualities of source and stored householdwaters in some selected communities in southwestern Nigeria. Sustainability (Switzerland), 9(3): 1–11.

BOSE, D. and KIME, M. M. (2019). Antimicrobial Susceptibility for Bacterial Isolates from Abattoir Effluent of Damaturu-Nigeria. International Journal of Advancement in Life Sciences Research, 2(2): 27–35.

CHAH, K. F., AGBO, I. C., EZE, D. C., SOMALO, S., ESTEPA, V. and TORRES, C. (2010). Antimicrobial resistance, integrons and plasmid replicon typing in multiresistant clinical Escherichia coli strains from Enugu State, Nigeria. Journal of Basic Microbiology, 50(1): 18-24.

CHAH, K. F., UGWU, I. C., OKPALA, A., ADAMU, K. Y., ALONSO, C. A., CEBALLOS, S., NWANTA J.A. and TORRES, C. (2018). Detection and molecular characterisation of extended-
spectrum β-lactamase-producing enteric bacteria from pigs and chickens in Nsukka, Nigeria. *Journal of Global Antimicrobial Resistance*, 15: 36-40

CLSI. (2017). CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th Ed. CLSI Supplement M100. Wayne, PA. p. 282.

EBIMIEOWEI, E. and IBEMOLOGI, A. (2016). Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. *International Journal of Applied Microbiology and Biotechnology Research*, 4: 90–101.

ELSAYED, T. I., ISMAIL, H. A., AHMED HA GAD, S. A. E. and GAD, A. H. (2017). The Occurrence of Multidrug Resistant E. Coli which Produce ESBL and Cause Urinary Tract Infections. *Journal of Applied Microbiology and Biotechnology*, 1(2): 90–101.

FAARI, AKANBI, A., FADEYI, A., WAHAB, K. and NWABUISI, C. (2015). Prevalence of extended spectrum beta-lactamase-producing Klebsiella species at the University of Ilorin Teaching Hospital. *Journal of Medical Investigations and Practice*, 10(1): 20-23.

FASHAE, K., LEEKITCHAROENPHON, P. and HENDRIKSEN, R. S. (2018). Phenotypic and genotypic comparison of salmonellae from diarrhoeic and healthy humans and cattle, Nigeria. *Zoonoses and Public Health*, 65(1): e185-e195

FOOUNOU, L., FOUNOU, R., NTSHOBENI, N., GOVINDEN, U., BESTER, L., CHENIA, H., DJOKO, C. and ESSACK, S. (2019). Emergence and Spread of Extended Spectrum β-Lactamase Producing Enterobacteriaceae (ESBL-PE) in Pigs and Exposed Workers: A Multicentre Comparative Study between Cameroon and South Africa. *Pathogens*, 8(1), 10.

FOOUNOU, L. L., FOUNOU, R. C. and ESSACK, S. Y. (2016). Antibiotic Resistance in the Food Chain: A Developing Country-Perspective. *Frontiers in Microbiology*, 7: 1881.

HILLE, K., RUDDAT, I., SCHMID, A., HERING, J., HARTMANN, M., VON MÜNCHHAUSEN, C., SCHNEIDER, B., MESSELHÄUSSER, U., FRIESE, A., MANSFELD, R., KÄSBOHRER, A., HÖRMANSDORFER, S., ROESLER, U and KREIENBROCK, L. (2017). Cefotaxime-resistant E. coli in dairy and beef cattle farms-Joint analyses of two cross-sectional investigations in Germany. *Preventive Veterinary Medicine*, 142: 39–45.

IKECHUKWU OKOYE, J., IZUCHUKWU ENE, G. and CHIJIOKE OJOBOR, C. (2016). Physico-Chemical and Microbiological Evaluation of Borehole Water Samples in Enugu, South-Eastern, Nigeria. *IOSR Journal of Environmental Science Ver. I*, 10(11): 2319–2399.

IROHA, I. R., ESIMONE, C. O., NEUMANN, S., MARLINGHAUS, L., KORTE, M., SZABADOS, F., GATERMANN, S. and KAASE, M. (2012). First description of Escherichia coli producing CTX-M-15- extended spectrum beta lactamase (ESBL) in out-patients from south eastern Nigeria. *Annals of Clinical Microbiology and Antimicrobials*, 11: 19.

JACOBY, G. A. (2005). Mechanisms of Resistance to Quinolones. *Clinical Infectious Diseases*, 41(Supplement_2): S120–S126.

LAWAL-ADEBOWALE, O. A. (2012). Dynamics of Ruminant Livestock Management in the Context of the
Nigerian Agricultural System. In Livestock Production, pg 61 - 80

MARKLAND, S., WEPPELMANN, T. A., MA, Z., LEE, S., MIR, R. A., TENG, L., GINN, A., LEE, C., UKHANOVA, M., GALINDO, S. … JEONG, K. C. (2019). High Prevalence of Cefotaxime Resistant Bacteria in Grazing Beef Cattle: A Cross Sectional Study. Frontiers in Microbiology, 10(2): 176.

MEDICINES DIRECTORATE, V. (2017). UK Veterinary Antibiotic Resistance and Sales Surveillance Report 2017. Retrieved from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/750811/_1473963v1-UK-VARSS_2017_Report_FINAL.pdf

MIR, R. A., WEPPELMANN, T. A., JOHNSON, J. A., ARCHER, D., MORRIS, J. G., JEONG, K. C. and JEONG, K. C. (2016). Identification and Characterization of Cefotaxime Resistant Bacteria in Beef Cattle. PloS One, 11(9): e0163279.

O’NEILL, J. (2014). Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. Retrieved from https://amr-review.org/sites/default/files/AMR Review Paper - Tackling a crisis for the health and wealth of nations_1.pdf

ODUYEBO, O., FALAYI, O., OSHUN, P. and ETTU, A. (2015). Phenotypic determination of carbapenemase producing Enterobacteriaceae isolates from clinical specimens at a tertiary hospital in Lagos, Nigeria. Nigerian Postgraduate Medical Journal, 22(4): 223.

OJO, O. E., SCHWARZ, S. and MICHAEL, G. B. (2016). Detection and characterization of extended-spectrum β-lactamase-producing Escherichia coli from chicken production chains in Nigeria. Veterinary Microbiology, 194:62 - 68

OKPARA, E. O., OJO, O. E., AWOYOMI, O. J., DIPEOLU, M. A., OYEKUNLE, M. A. and SCHWARZ, S. (2018). Antimicrobial usage and presence of extended-spectrum β-lactamase-producing Enterobacteriaceae in animal-rearing households of selected rural and peri-urban communities. Veterinary Microbiology, 218(10): 31–39.

OLI, A.N., OGBUAGU, V.I., EJIKEUGWU, C.P., IROHA, I.R., UGWU, M. C., I.,
OFOMATA, C.M., OKEKE, K.N., ... and ONAH, S.K. (2019). Multi-Antibiotic Resistance and Factors Affecting Carriage of Extended Spectrum β-Lactamase-Producing Enterobacteriaceae in Pediatric Population of Enugu Metropolis, Nigeria. *Medical Sciences*, 7(11): 104.

OLOSO, N. O., FAGBO, S., GARBATI, M., OOLONITOLA, S. O., AWOSANYA, E. J., AWORH, M. K., ... and FASINA, F. O. (2018). Antimicrobial resistance in food animals and the environment in Nigeria: A review. *International Journal of Environmental Research and Public Health*, 15(6):1284.

OLOWE, O. A., ADEWUMI, O., ODEWALE, G., OJURONGBE, O. and ADEFOYE, O. J. (2015). Phenotypic and Molecular Characterisation of Extended-Spectrum Beta-Lactamase Producing Escherichia coli obtained from Animal Fecal Samples in Ado Ekiti, Nigeria. *Journal of Environmental and Public Health* 2015:e497980.

OLOWE, O. A., CHOUDHARY, S., SCHIERACK, P., WIELER, L. H., MAKANJUOLA, O. B., OLAYEMI, A. B. and ANJUM, M. F (2013). Pathotyping bla CTX-M Escherichia coli from Nigeria. *European Journal of Microbiology & Immunology*, 3(2): 120–125.

OLOWO-OKERE, A., IBRAHIM, Y. K. E. and OLAYINKA, B. O. (2018). Molecular characterisation of extended-spectrum β-lactamase-producing Gram-negative bacterial isolates from surgical wounds of patients at a hospital in North Central Nigeria. *Journal of Global Antimicrobial Resistance*, 14:85 - 89.

OMOSHABA, E. O., OLUFEMI, F. O., OJO, O. E., SONIBARE, A. O. and AGBAJE, M. (2017). Multidrug-resistant Salmonellae isolated in Japanese quails reared in Abeokuta, Nigeria, 49(7): 1455 - 1460.

ONWUGHARA, N. I., AJIWE, V. E., NNABUENI, H. O. and CHIMA, C. H. (2013). Bacteriological Assessment of Selected Borehole Water Samples in Umuahia North Local Government Area, Abia State, Nigeria. In *Journal of Environmental Treatment Techniques*, 1(2): 117 - 121.

PÉREZ, C. D.-A., LÓPEZ-FRESNEÑA, N., CARLAVILLA, A. L. R., GARCIA, M. H., RUIZ-GARBAJOSA, P., ARANAZ-ANDRÉS, J. M., ... and CANTON, R. (2019). Local prevalence of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae intestinal carriers at admission and co-expression of ESBL and OXA-48 carbapenemase in Klebsiella pneumoniae: a prevalence survey in a Spanish University Hospital. *BMJ Open*, 9(3): e024879.

SANDHU, R. (2016). Evaluation of multiple antibiotic resistance (MAR) index and Doxycycline susceptibility of Acinetobacter species among inpatients. *International Journal of Infectious Diseases*, 45:327.

THIPPESWAMY, J., PATHAK, A., PATIL, C., SAIKIA, R., CHOUDHURY, S., & SHUKLA, A. (2018). Doping in animals:: A concise outlook. *International Journal of Science, Environment and Technology*, 7(4): 1427–1430.

VELASOVA, M., SMITH, R. P., LEMMA, F., HORTON, R. A., DUGGETT, N. A., EVANS, J., ... and RANDALL, L. P. (2019). Detection of extended-spectrum β-lactam, AmpC and carbapenem resistance in Enterobacteriaceae in beef cattle in Great Britain in 2015. *Journal of Applied Microbiology*, 126(4): 1081–1095.
WERNLI, D., JØRGENSEN, P. S., HARBARTH, S., CARROLL, S. P., LAXMINARAYAN, R., LEVRAT, N., …and PITTET, D. (2017). Antimicrobial resistance: The complex challenge of measurement to inform policy and the public. *PLOS Medicine, 14*(8): e1002378.

WHO. (2014). Antimicrobial resistance: global report on surveillance. In *World Health Organization Report*. pp 256

WHO. (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. *Who, 7*. Retrieved from https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

WHO. (2018). Nigeria Risk of Premature Death due to Non-communicable Diseases. Retrieved August 24, 2020, from World Health Organization-Noncommunicable Disease Country Profile website: https://www.who.int/nmh/countries/nga_en.pdf?ua=1.