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Prevalence and Antimicrobial Resistance in *Escherichia coli* from Food Animals in Lagos, Nigeria

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Foodborne bacteria are often associated with human infections; these infections can become more complicated to treat if the bacteria are also resistant to antimicrobials. In this study, prevalence, antimicrobial resistance, and genetic relatedness of *Escherichia coli* among food producing animals from Lagos, Nigeria, was investigated. From December 2012 to June 2013, *E. coli* were isolated from fecal samples of healthy cattle, chicken, and swine. Antimicrobial susceptibility testing against 22 antimicrobials was performed using broth microdilution with the Sensititre™ system. Clonal types were determined by pulsed-field gel electrophoresis (PFGE). From the analysis, 211/238 (88.7%), 170/210 (81%), and 136/152 (89.5%) samples from cattle, chicken, and swine, respectively, were positive for *E. coli.* A subset of those isolates ($n=211$) selected based on β-lactamase production was chosen for further study. Overall, *E. coli* exhibited the highest resistance to tetracycline (124/211; 58.8%), trimethoprim/sulfamethoxazole (84/211; 39.8%), and ampicillin (72/211; 34.1%). Approximately 40% of the isolates were pan-susceptible, and none of the isolates were resistant to amikacin, cefepime, ceftazidime, ertapenem, meropenem, or tigecycline. Among the resistant isolates, 28 different resistance patterns were observed; 26 of those were characterized as multi-drug resistant (MDR; resistance to ≥ 2 antimicrobials). One isolate was resistant to 13 different antimicrobials representing five different antimicrobial classes. Using PFGE, MDR *E. coli* were genetically diverse and overall did not group based on source; identical PFGE patterns were detected among isolates from different sources. These results suggest that isolates cannot be attributed to specific sources, and some may be present across all of the sources. Results from this study indicate that food-producing animals in Nigeria are a reservoir of MDR *E. coli* that may be transferred to humans via the food chain.

Introduction

Antimicrobial drugs have played a major role in the therapy of bacterial infections by reducing morbidity and mortality associated with infectious diseases in humans and animals. However, antimicrobial resistance in bacteria, particularly those isolated from food animals, has increased worldwide.[11,19,31] This increase has been attributed to a number of factors, including misuse of antimicrobial drugs in humans and animals, the addition of antimicrobials to livestock feeds, and use of antimicrobial drugs for growth promotion.[1] Food-producing animals have been documented as a reservoir of resistant bacteria, and products from those animals have been documented as a source of foodborne infections in humans.[12,13,33]

Escherichia coli is an intestinal commensal bacterium of humans and animals and has been frequently associated with foodborne illness.[13] *E. coli* can be opportunistic or true pathogens causing illnesses such as intestinal and extra-intestinal infections, urinary tract infections, gastro-intestinal infections, meningitis, peritonitis, and septicemia.[9,16,18] Both commensal and pathogenic strains acquire antimicrobial resistance and have been implicated in the transfer of resistance to other bacteria, including other pathogenic bacteria, in the environment and within animal sources.[20,36] *E. coli* may be spread to humans via the food chain during slaughter of the animals, through improper handling of food, or inadequate cooking.[11,13]

Although there are a number of studies on antimicrobial resistance in *E. coli* from food animals in the western world, there is a paucity of data on prevalence of multi-drug resistant (MDR; resistance to ≥ 2 antimicrobials) *E. coli* from food animals in developing countries such as Nigeria.[4,6,21,24] This information is useful to compare trends in resistance in countries where antimicrobial use in both humans and animals is poorly regulated. In Nigeria, the major sources of

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meat consumed are beef and poultry and food-producing animals serve as major sources of meat to the population of Lagos State, Nigeria, which currently comprises more than 21 million people (www.lagosstate.nig). Previous studies on prevalence and antimicrobial resistance of commensal *E. coli* from food animals from different parts of Nigeria have reported high levels of resistance.[4,6,21,24] Of particular concern is resistance to antimicrobials such as aminoglycosides, cephalosporins, fluoroquinolones, and sulfonamides that are commonly used to treat human infections caused by Gram-negative bacteria.[11] Furthermore, MDR *E. coli* from food animals are prevalent in Nigeria as reported in one study where 71 different MDR types were identified in *E. coli* from cattle.[4]

Although prevalence of commensal *E. coli* and their antimicrobial resistance patterns were determined in previous studies,[4,6,21,24] few of those studies included *E. coli* from all major meat animals consumed in Nigeria and none tested the isolates against a wide range of antimicrobials primarily used for treating human infections. The purpose of this study was to determine prevalence and antimicrobial resistance of *E. coli* isolated from chicken, cattle, and swine in Lagos, Nigeria. In addition, the genetic relatedness among the isolates was also studied to determine whether genetic similarities exist among antimicrobial resistant *E. coli* from the three food animal groups.

Materials and Methods

Bacterial isolates

From December 2012 to June 2013, fecal samples ($n = 600$) were collected from food-producing animals in Lagos State, Nigeria. Voided chicken fecal samples were collected from coops and cages from six farms and six central open markets where farmers transport and sell cocks and broilers to retailers. All cattle tested were free-grazing before they were taken to the abattoir where they were kept in cages and sheds before slaughter. Cattle samples were from two abattoirs, the central abattoir for the whole of Lagos State found on the mainland of Lagos and an abattoir in a semi-urban community. Fecal samples were collected from the lower intestine and rectum of the cattle during slaughter. Pigs were reared on family-owned farms and sold to retailers at a central abattoir when they were ready for slaughter. Fecal samples from the lower intestine and rectum of the pigs were collected from two piggeries and from the abattoir at slaughter. Three to four pigs were housed in pens or cages depending on the size of the animal. Locations of sampled abattoirs, poultries, and piggeries are indicated on the map of Lagos (Fig. 1). Ethical approval for sampling was obtained from the Nigerian Institute of Medical Research (NIMR) Institutional Review Board and Lagos State Agricultural Service Commission. Samples were collected with sterile swabs that were premoistened with phosphate-buffered saline (1×) and kept on ice. All samples were processed within 4 hr of collection. All samples were cultivated on MacConkey and 5% sheep blood agar (Oxoid, Basingstoke, United Kingdom) for the presence of members of the *Enterobacteriaceae* and were incubated aerobically for 24 hr at 37°C.[2,3] One to two colonies were selected from each sample. Isolates were Gram-stained and identified using API 20E according to the manufacturer's directions (bioMérieux, Basingstoke, United Kingdom). *E. coli* were identified using the VITEK®2 System and the VITEK 2GN identification card (bioMérieux, Durham, NC) according to the manufacturer's directions. A subset of *E. coli* was selected for study by their ability to produce β-lactamases using a β-lactamase test strip (bioMérieux). All bacterial strains were stored at −80°C in brain heart infusion broth with 30% glycerol.

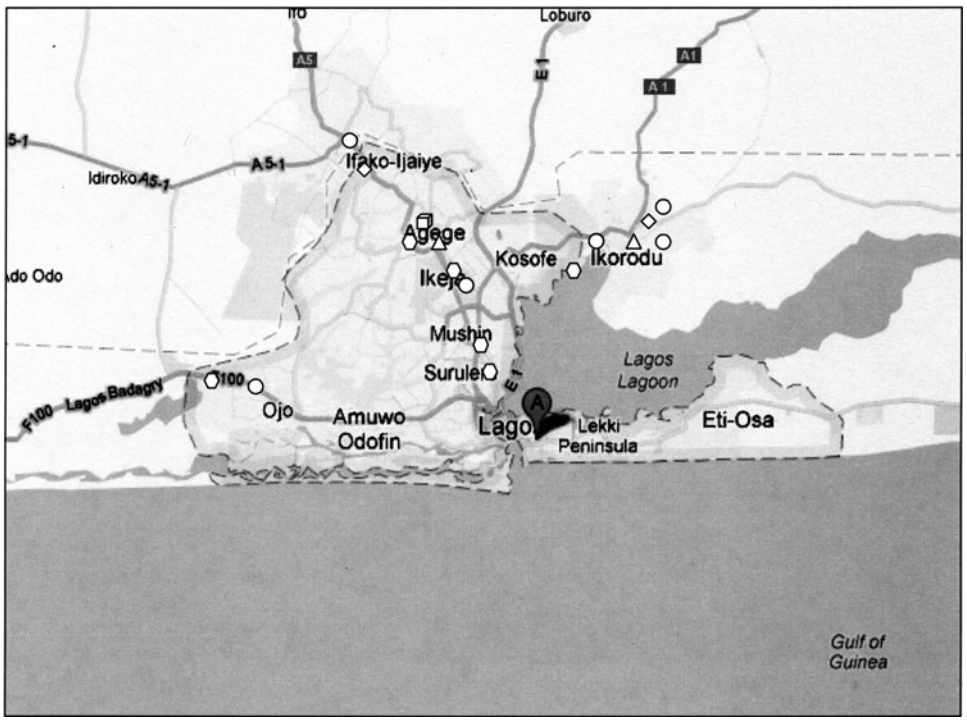

FIG. 1. Map of collection sites for cattle, chicken, and swine samples. Farms were located within a range of 10–30 km. *Cubed boxes* represent abattoir (swine); *triangles* represent abattoir (cattle); *hexagons* represent markets for chicken; *circles* represent poultries; and *diamonds* represent piggeries.

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MIC, μg/ml) for *E. coli* were determined by broth microdilution using the Sensititre semi-automated susceptibility system (TREK Diagnostic Systems, Inc., Westlake, OH) and the Sensititre Gram-negative plate GN3F according to the manufacturer's directions. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI)[7] with the exception of tigecycline and cephalothin. A breakpoint for resistance of tigecycline to *Enterobacteriaceae* has not been defined; for this study, the MIC for tigecycline was ≥1 μg/ml. The breakpoint for cephalothin as defined by CLSI is ≥32 μg/ml; however, the highest concentration of cephalothin on the susceptibility plate was 16 μg/ml. Therefore, the breakpoint used for determining resistance to cephalothin in this study was defined as >16 μg/ml. Antimicrobials and breakpoints (in parenthesis) were as follows: amikacin (≥64 μg/ml), ampicillin (≥32 μg/ml), ampicillin/sulbactam (≥32/16 μg/ml), aztreonam (≥16 μg/ml), cefazolin (≥8 μg/ml), cefepime (≥32 μg/ml), cefoxitin (≥32 μg/ml), cefpodoxime (≥8 μg/ml), ceftazidime (≥16 μg/ml), ceftriaxone (≥4 μg/ml), cefuroxime (≥32 μg/ml), cephalothin (>16 μg/ml), ciprofloxacin (≥4 μg/ml), ertapenem (≥2 μg/ml), gentamicin (≥16 μg/ml), meropenem (≥4 μg/ml), piperacillin/tazobactam (≥128/4 μg/ml), tetracycline (≥16 μg/ml), ticarcillin/clavulanic acid (≥128/2 μg/ml), tobramycin (≥16 μg/ml), trimethoprim/sulfamethoxazole (≥4/76 μg/ml), and tigecycline (≥1 μg/ml). *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 29213 were controls for determination of MIC.

Pulsed-field gel electrophoresis

A 24 hr *E. coli* pulsed-field gel electrophoresis (PFGE) procedure was performed as previously described.[29] Briefly, cells from an overnight culture were embedded in 1.0% Seakem Gold agarose (BioWhittaker Molecular Applications, Rockland, ME) and digested with 10 U of *Xba*I (Roche Molecular Biochemicals, Indianapolis, IN). DNA standards were prepared from *Salmonella enterica* serotype Braenderup H9812. Digested DNA was separated using the CHEF-DRII PFGE system as per manufacturer's instructions (Bio-Rad, Hercules, CA). Electrophoresis was performed at 6 V for 19 hr with a ramped pulse time of 2.16–54.17 sec in 0.5×Tris-borate-EDTA (TBE) at14°C. Cluster analysis was determined using BioNumerics software (Applied Maths Scientific Software Development, Sint-Martens-Latem, Belgium) using Dice coefficient and the unweighted pair-group method (UPGMA). Optimization settings for dendrograms were 1.5% with a position tolerance of 1.5%. Isolates with ≥66% similar profiles were considered to represent the same clone.[35]

Results

Prevalence and antimicrobial resistance of E. coli

Six hundred fecal samples from cattle ($n=238$), chicken ($n=210$), and swine ($n=152$) were collected and tested for the presence of *E. coli*. Of those, swine had the highest number of positive samples (89.5%; 136/152), followed by cattle (88.7%; 211/238) and chicken (81%; 170/210) (Table 1). Since the majority of antimicrobials on the susceptibility plate used in this study were β-lactams, *E. coli* selected for further study ($n=211$) were those positive for β-lactamase production.

E. coli were resistant to 16 of the 22 antimicrobials tested (Table 2). From all sources, resistance to tetracycline (59%; 124/211) was the highest, followed by trimethoprim/sulfamethoxazole (40%; 84/211) and ampicillin (34.1%; 72/211). With the exception of ampicillin, overall resistance to other β-lactams, including ampicillin/sulbactam, aztreonam, cefazolin, cefoxitin, cefpodoxime, ceftriaxone, cefuroxime, cephalothin, piperacillin/tazobactam, and ticarcillin/clavulanic acid, was low (less than 20%). None of the isolates from any of the sources were resistant to several additional β-lactams tested, including cefepime, ceftazidime, ertapenem, and meropenem (Table 2). Of the three aminoglycosides tested, only resistance to gentamicin and tobramycin was observed; none of the isolates were resistant to amikacin (Table 2). Resistance in *E. coli* from chickens to gentamicin (20%; 16/80) and tobramycin (2.9%; 3/103) was higher in chickens than in cattle (2.9%, 3/103 and 1%; 1/103, respectively); all swine isolates were susceptible to both gentamicin and tobramycin. Approximately 40% (85/211) of the isolates were pan-susceptible.

Among the resistant *E. coli*, 45.5% (96/211) were MDR and exhibited 26 different MDR patterns (Table 3). The most diversity was observed in MDR *E. coli* from chickens that exhibited 21 MDR patterns. Ten MDR patterns were observed in MDR *E. coli* from cattle, and only four were observed in swine. MDR *E. coli* were resistant to as few as two and as many as 13 antimicrobials. The majority of isolates ($n=11$) were resistant to three different classes of antimicrobials; one isolate from chickens was resistant to five different classes (Table 3). The most common resistance pattern ($n=39$) was AmpTetSxt found in *E. coli* from cattle and chickens. The highest number of MDR isolates was from chickens (49/80; 61.3%) followed by cattle (40.8%; 42/103); very little MDR *E. coli* were isolated from swine (17.9%; 5/28).

Genetic relatedness

MDR *E. coli* ($n=96$) were subjected to PFGE analysis to determine genetic relatedness (Fig. 2). Using PFGE analysis, various clustering was examined according to PFGE pattern, antimicrobial resistance, or source. Eight clusters (A-H) were identified that were ≥66% similar. Six clusters were composed of isolates from at least two sources (clusters A, B, C, E, G, and H), whereas two clusters (clusters D and F) contained isolates from all three sources. Cluster A was almost entirely composed of MDR *E. coli* from chickens. Three of

Table 1. Prevalence of *Escherichia coli* Among Food-Producing Animals

Source	*No. samples* (n=*600*)	*No. positive samples (%)*[a]	*No.* E. coli *isolates*
Cattle	238	211 (88.7)	103
Chicken	210	170 (81)	80
Swine	152	136 (89.5)	28

[a]Isolates selected for inclusion in this study were positive for β-lactamase production.

Table 2. Antimicrobial Resistance of *Escherichia coli* Isolated from Food Animals

Antimicrobial[a]	*Breakpoint (μg/ml)*	*No. resistant (%)*		
		Cattle (n = 103)	*Chicken (n = 80)*	*Swine (n = 28)*
Ampicillin	> 32	36 (35)	33 (41.3)	3 (10.7)
Ampicillin/sulbactam	≥ 32/16	5 (4.9)	9 (11.3)	3 (10.7)
Aztreonam	≥ 16	0 (0)	5 (6.3)	1 (3.6)
Cefazolin	≥ 8	1 (1)	5 (6.3)	1 (3.6)
Cefoxitin	≥ 32	1 (1)	0 (0)	0 (0)
Cefpodoxime	≥ 8	0 (0)	5 (6.3)	1 (3.6)
Ceftriaxone	≥ 4	0 (0)	5 (6.3)	1 (3.6)
Cefuroxime	> 32	0 (0)	5 (6.3)	1 (3.6)
Cephalothin	> 16	1 (1)	6 (7.5)	2 (7.1)
Ciprofloxacin	≥ 4	2 (1.9)	8 (10)	1 (3.6)
Gentamicin	≥ 16	3 (2.9)	16 (20)	0 (0)
Piperacillin/tazobactam	≥ 128/4	0 (0)	1 (1.3)	0 (0)
Tetracycline	≥ 16	52 (50.5)	58 (72.5)	14 (50)
Ticarcillin/clavulanic acid	≥ 128/2	1 (1)	3 (3.8)	0 (0)
Tobramycin	> 16	1 (1)	6 (7.5)	0 (0)
Trimethoprim/sulfamethoxazole	≥ 4/76	38 (36.9)	41 (51.3)	5 (17.9)

[a]No isolates were resistant to amikacin, cefepime, ceftazidime, ertapenem, meropenem, or tigecycline.

Table 3. Single and Multidrug Resistance Patterns in *Escherichia coli* from Food Animals

Pattern	*No. of resistances*	*No. of resistance by class*	*No. of isolates per animal*		
			Cattle (n = 103)	*Chicken (n = 80)*	*Swine (n = 28)*
Pan-susceptible	0	0	51	20	13
Sxt	1	1	0	0	1
Tet	1	1	10	11	9
AmpGen	2	2	0	1	0
AmpSxt	2	2	0	1	0
AmpTet	2	2	3	2	0
CipTet	2	2	0	2	0
TetSxt	2	2	5	6	2
AmpA/Stet	3	2	1	0	0
AmpTetSxt	3	3	27	12	0
CipTetSxt	3	3	0	1	0
GenTetSxt	3	3	0	2	0
GenTetTob	3	2	0	2	0
AmpGenTetSxt	4	4	1	5	0
AmpA/SCepTet	4	2	0	0	1
AmpCipTetSxt	4	4	0	1	0
AmpA/STetSxt	4	3	0	2	0
AmpA/STetTim	4	2	0	1	0
AmpA/SCepTetSxt	5	3	0	1	0
AmpA/SCipTetSxt	5	4	1	0	1
AmpA/SGenTetSxt	5	4	1	2	0
AmpA/STetTimSxt	5	3	1	0	0
CipGenTetTobSxt	5	4	1	3	0
AmpA/SFazFoxCepTetSxt	7	3	1	0	0
AmpAztFaxPodAxoFurCepTetSxt	9	3	0	1	0
AmpA/SAztFazPodAxoFurCepTetSxt	10	3	0	1	1
AmpAztFazPodAxoFurCepTetTimSxt	10	3	0	1	0
AmpA/SAztFazPodAxoFurCepP/T4TetTimSxt	12	3	0	1	0
AmpA/SAztFazPodAxoFurCepCipGenTetTobSxt	13	5	0	1	0

Amp, ampicillin; A/S, ampicillin/sulbactam; Axo, ceftriaxone; Azt, aztreonam; Cep, cephalothin; Cip, ciprofloxacin; Faz, cefazolin; Fox, cefoxitin; Fur, cefuroxime; Gen, gentamicin; P/T4, piperacillin/tazobactam; Pan-susceptible, susceptible to all tested antimicrobials; Pod, cefpodoxime; Tet, tetracycline; Tim, ticarcillin/clavulanic acid; Tob, tobramycin; Sxt, trimethoprim/sulfamethoxazole.

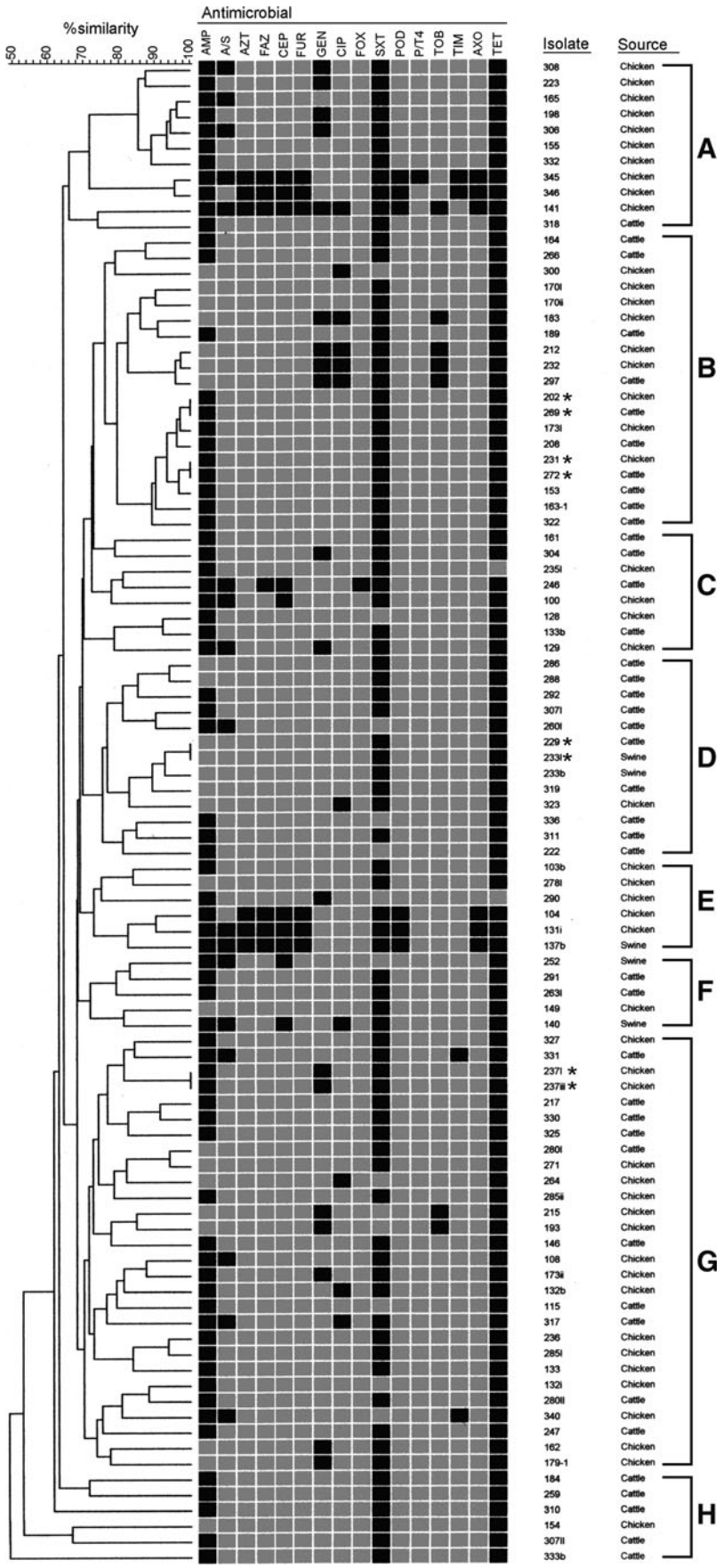

FIG. 2. Pulsed-field gel electrophoresis (PFGE) analysis and antimicrobial resistance patterns of multi-drug resistant (MDR; resistance to ≥2 antimicrobials) *Escherichia coli* from cattle, chickens, and swine. DNA for PFGE was digested with *Xba*I. Levels of similarity were determined using Dice coefficient and the unweighted pair group method (UPGMA). Clusters were based on ≥66% similarity and are labelled **(A–H).** *Black boxes* represent resistance; *gray boxes* represent susceptible or intermediate. *Asterisks* indicate isolates with identical PFGE patterns. Antimicrobials are ampicillin (AMP), ampicillin/sulbactam (A/S), aztreonam (AZT), cefazolin (FAZ), cephalothin (CEP), cefuroxime (FUR), gentamicin (GEN), ciprofloxacin (CIP), cefoxitin (FOX), trimethoprim/sulfamethoxazole (SXT), cefpodoxime (POD), piperacillin/tazobactam (P/T4), tobramycin (TOB), ticarcillin/clavulanic acid (TIM), ceftriaxone (AXO), and tetracycline (TET). No isolates were resistant to amikacin, cefepime, ceftazidime, ertapenem, meropenem, or tigecycline.

the *E. coli* from chickens in this cluster displayed resistance to a greater number of antimicrobials than the rest of the isolates (10–13 antimicrobials) (Fig. 2). Grouping of *E. coli* resistant to greater numbers of antimicrobials was also observed in cluster E. Although only 6 isolates were present in this cluster, 2 were resistant to 10 antimicrobials (1 isolate from chickens and 1 from swine), whereas one *E. coli* from chickens was resistant to nine antimicrobials. All three were resistant to the same nine antimicrobials.

Cluster G contained the greatest number of isolates ($n=28$) from cattle and chickens. Two isolates (237i and 237ii) originating from chickens were 100% identical according to the PFGE pattern and were also resistant to the same antimicrobials (Fig. 2). Although isolates that were 100% identical were not predominant using PFGE, three other sets of isolates also had identical PFGE patterns. Cluster B contained the second most number of isolates ($n=19$) and two sets of isolates with identical PFGE and antimicrobial susceptibility patterns. Isolates 202 and 269 originated from chicken and cattle, respectively, but were 100% identical in PFGE patterns and were resistant to AmpTetSxt (Fig. 2). Similarly, isolates 231 and 272 also shared the same PFGE pattern and resistance to AmpTetSxt, but originated from chicken and cattle, respectively. The only other cluster containing isolates with identical PFGE patterns was cluster D, which contained 13 MDR *E. coli* from cattle, chickens, and swine (Fig. 2). Isolates 229 and 233i were from cattle and swine, respectively, and were resistant to only two antimicrobials, trimethoprim/sulfamethoxazole, and tetracycline. Isolates in cluster H were the least similar to other isolates as well as to each other, although they were resistant to the most common MDR phenotype in this study (AmpTetSxt).

Discussion

The prevalence of antimicrobial resistance and MDR *E. coli* among clinical isolates has been studied in some regions of Nigeria.[2,22] The results of the studies have been a cause of concern for clinicians due to the high level of antimicrobial resistance among the isolates tested. However, there is a paucity of data on prevalence and antimicrobial resistant *E. coli* from food-producing animals that may also have a role in the dissemination of antimicrobial resistance among humans in Nigeria.[4,6,15,21,26] This information is essential to determine the possibility of spread of antimicrobial resistance to the human population from healthy food-producing animals at slaughter or for sale to the public. In this study, the prevalence of *E. coli* from healthy cattle, chickens, and swine and antimicrobial resistance profiles, including MDR patterns among the isolates, was determined. Genetic analysis of the isolates also allowed common genetic types among the different food-producing animal groups to be identified.

From the susceptibility results of this study, resistance to tetracycline was the most prevalent. This was not surprising, as resistance to tetracycline in *E. coli* from poultry in Nigeria has been reported as early as 1985.[23] Recent reports from other prevalence studies of *E. coli* from food-producing animals from Nigeria have estimated tetracycline resistance ranging from 64 to 72%.[4,21,26] These results are consistent with other studies from around the world, including the animal arm of the National Antimicrobial Resistance Monitoring System (NARMS) (www.ars.usda.gov/SP2UserFiles/Place/66120508/NARMS/NARMS2009/Table 3C.pdf). The observation that resistance to tetracycline was elevated among the isolates may be attributed to the use of tetracycline by farmers in treating food animals in Nigeria, most likely due to the cheap rate at which it is sold, easy availability of the drug, as well as long-term use in animals and humans. Tetracycline is commonly sold over the counter or vended by medicine sellers in the Nigerian community.

Although the second highest level of resistance in this study was to ampicillin, other studies on commensal and shiga-toxin producing *E. coli* from Nigeria have reported much higher levels of ampicillin resistance (up to 85%).[4,21,24] Susceptibility results for other β-lactams from this study also contrasted greatly with results from other studies where higher levels of resistance to other β-lactams such as cefoxitin, ceftriaxone, cephalothin, and cefpodoxime were observed.[4,21,24] This could be due to the method of susceptibility testing used (disc diffusion versus microbroth dilution) or the geographic area where the samples were taken. β-lactam antimicrobials are very useful for treatment of Gram-negative bacterial infections in humans, and increasing resistance to those drugs may indicate that they will not be available for treatment of those infections in humans in the future.[11] The lack of resistance to the carbapenems, ertapenem and meropenem, was encouraging as carbapenems are usually the last-line defense for treatment of infections caused by Gram-negative bacteria.[28] Susceptibility results using these drugs have not previously been available for *E. coli* isolates in Nigeria.

The level of resistance to gentamicin in chicken was unexpected. Gentamicin resistance for chickens in this study was comparable to previous reports, although levels as high as 88% have been reported for cattle.[4] In the United States, aminoglycosides may be used to control *Salmonella* infections in cattle and to prevent or treat *Salmonella* or *E. coli* infections in poults.[11,30] However, since aminoglycosides are toxic in nature and meat-holding times are extended due to drug residues, their use is limited in food animal production in the United States.[11] Although aminoglycoside use in poultry or cattle production in Nigeria is unknown, none of the *E. coli* isolates were resistant to the newer aminoglycoside, amikacin.

MDR *E. coli* in food animals in Nigeria appears to be prevalent.[4,6,21,24,26] Although less than 50% of the isolates in this study were MDR, 94% MDR *E. coli* have been observed in poultry in Nigeria.[26] This may be due to the different antimicrobials used in this study, as many were antimicrobials primarily used in treating infections in humans; whereas other studies included older antimicrobials and antimicrobials used in both human and animal medicine. The difference in antimicrobials tested was also reflected in the high number of pan-susceptible *E. coli* in this study. Furthermore, the number of different patterns found and the most common number of antimicrobials that the isolates were resistant to were also very high, which may result in treatment failure even with the use of multiple antimicrobials.[34] Although 26 different MDR patterns were observed in this study, some studies have found much higher numbers of MDR patterns.[4,21,26] MDR to three to four antimicrobials appears to be the most common among MDR *E. coli* from this study and others.[4,26] MDR *E. coli* may also be a result of indiscriminate use of antibiotics in the feeds and

water fed to the animals that has been observed in food-producing animal farms.[10,25] Similarly, other studies have shown that antimicrobials have been added to feeds to promote growth and for treating infections in developed countries.[17,37] This could account for MDR, which was observed among all three animal sources in this study.

Antimicrobial resistance of *E. coli* isolates in chicken has been linked to extra–intestinal human infections and avian pathogenic strains.[9] Interestingly, this study observed that chicken had the highest number of MDR *E. coli* isolates followed by cattle and then swine isolates. This is in contrast to a report using fecal samples from newborn piglets in which 65% of samples were positive for *E. coli* that was resistant to at least one antimicrobial tested.[5] In the Nigerian community, farmers add antimicrobials to chicken feed to prevent infections on farms, invariably increasing the size of the chicken to boost sales, as chicken is a staple meat product.[25] The practice is rather less common in piggeries, which may be one reason that MDR was lower in *E. coli* from swine. Compared with beef and chicken, there is a lower consumption of pork in some parts of Nigeria partly due to religious reasons contributing to less swine production in the country. MDR among cattle isolates in this study was much lower than that in a previous report in which a high prevalence of MDR among *E. coli* isolates from fecal samples of cattle was found.[4] The study noted the use of tetracycline as a replacement diet or additive in milk for young animals as a probable cause of drug resistance. Nomadic cattle grazing is a common practice in Africa; unrestricted close contact between cattle and the herdsmen is frequent. Animals may be exposed to excreta from humans and possibly immuno-compromised handlers because of the unique exposure of the herdsmen. A horizontal antimicrobial resistance transfer through the fecal-oral route is a possibility among this group of animals.[13,14,27] Furthermore, the cattle are given water to drink from different communities while traversing from one region to the other; therefore, they are also exposed to different sources of water, including wells, streams, and rivers, which may be a reservoir of resistant organisms.[32]

PFGE is considered the ''gold-standard'' for molecular typing of most bacteria and provides consistent results, as the method has been standardized and is an efficient tool for epidemiological investigations.[29,35] PFGE analysis of the MDR *E. coli* revealed a genetically diverse population; high genetic diversity among animal *E. coli* has been previously reported.[21] All of the clusters identified contained isolates from all three sources, suggesting that isolates are not specific for a particular source and a single clone is not circulating among the food animals. However, four groups of isolates shared 100% identical banding patterns and isolates from three of those groups were from different sources. Identical isolates within the four groups were also resistant to the same antimicrobials. It is of note that some of the isolates that were in the same cluster had different antimicrobial phenotypes, suggesting that isolates with similar PFGE types do not necessarily have the same antimicrobial phenotypes.[8] Taken together, these data suggest that although the isolates are genetically diverse, some may be shared among the different food animal sources which could be the result of environmental or other cross-contamination events.

This study indicates that food-producing animals in Lagos, Nigeria, may serve as a reservoir of antimicrobial-resistant commensal *E. coli* that can be disseminated to the community. Therefore, there is a critical need to monitor the use of antimicrobials in animals and animal feeds and establish surveillance on animal products sold in the markets, especially to clinically relevant antimicrobials essential for use in treating human bacterial infections. This study has determined that antimicrobial-resistant commensal *E. coli* is prevalent among food animals in Lagos and that these isolates are also MDR, indicating that they may harbor resistance genes that may be transferred to humans via the food chain. Additional studies on identifying those resistance genes and their ability to mobilize are needed.

Disclosure Statement

The mention of trade names or commercial products in this manuscript is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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