Antibiotic resistance profiles among mesophilic aerobic bacteria in Nigerian chicken litter and associated antibiotic resistance genes[1]

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ABSTRACT The effect of global antibiotic use practices in livestock on the emergence of antibiotic resistant pathogens is poorly understood. There is a paucity of data among African nations, which suffer from high rates of antibiotic resistant infections among the human population. *Escherichia* (29.5%), *Staphylococcus* (15.8%), and *Proteus* (15.79%) were the dominant bacterial genera isolated from chicken litter from four different farms in Zaria, Nigeria, all of which contain human pathogenic members. *Escherichia* isolates were uniformly susceptible to augmentin and cefuroxime, but resistant to sulfamethoxazole (54.5%), ampicillin (22.7%), ciprofloxacin (18.2%), cephalothin (13.6%) and gentamicin (13.6%). *Staphylococcus* isolates were susceptible to ciprofloxacin, gentamicin, and sulfamethoxazole, but resistant to tetracycline (86.7%), erythromycin (80%), clindamycin (60%), and penicillin (33.3%). Many of the isolates (65.4%) were resistant to multiple antibiotics, with a multiple antibiotic resistance index (MARI) $\geq$ 0.2. *sul*1, *sul*2, and *van*A were the most commonly detected antibiotic resistance genes among the isolates. Chicken litter associated with antibiotic use and farming practices in Nigeria could be a public health concern given that the antibiotic resistant patterns among genera containing pathogens indicate the potential for antibiotic treatment failure. However, the MARI values were generally lower than reported for *Escherichia coli* from intensive poultry operations in industrial nations.

Key words: antibiotics, antibiotic resistance, chicken, MARI, Nigeria

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INTRODUCTION

Antibiotic resistance is one of the greatest human health challenges of our time and the case has been made that the world is dangerously close to a return to the pre-antibiotic era (Chander et al., 2007). There is growing concern and evidence (Rinsky et al., 2013) that agricultural antibiotic use may be contributing significantly to increased rates of antibiotic resistance. In many countries, the majority of antibiotics used are administered to livestock, and in the United States and many countries the majority is for prophylactic purposes or to stimulate growth promotion. Of particular concern are antibiotics that are used both in human and livestock medicine, such as third generation cephalosporins (Collignon et al., 2013), though even use of distinct antibiotics poses a risk for cross-selection or coselection of resistance to human antibiotics (Hedges and Shannon, 1984) and can stimulate horizontal gene transfer [i.e., transfer of antibiotic resistance genes (**ARG**) among bacteria] (Gillings and Stokes, 2012).

Livestock populations have been found to be reservoirs of several critical antibiotic resistant human pathogens, including vancomycin-resistant *Enterococci* (Bates, 1997). In many other countries and much of the developing world, the use of antibiotics in livestock is unregulated and a prescription is not required even for human use (Hart and Kariuki, 1998). In general, antibiotic use in livestock in Africa (Siwela et al., 2007) is unrestricted. Thus, antibiotic use practices vary widely across the globe and there is an opportunity to learn about their general effects by comparing antibiotic resistance patterns among livestock from different regions of the world. In particular, although much effort has been directed at improving the poor circumstances of public health in Africa, little is known about the status of antimicrobial resistance in African livestock populations or potential linkages to human illness.

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Poultry are of particular interest given their reported linkage with vancomycin-resistant *Enterococci* infections in humans in European studies (Hayes et al., 2004). Unlike the intensive poultry farming as practiced essentially in urban settings, "free range" farming is the major form in the rural and peri-urban areas of Nigeria where modern facilities such as electricity supplies are not available, and is usually intended for local consumption (Ajala et al., 2008). It involves extensive rearing of birds around households on a small to medium scale basis, allows for extensive movement (outside of nonconfining enclosures) and exposure to sunlight, and requires affordable capital inputs since the birds feed mainly by scavenging. Also local breeds of bird (*Gallus domesticus*) are mostly used. The turnover rates are high and the protein needs of the poor households are partially met. It is also thought to be a relatively sustainable and cost effective practice. Large scale poultry farming is usually practiced in urban areas where available lands for extensive rearing of birds are lacking (Kperegbeyi et al., 2009). This practice is also sometimes called commercial or industrial poultry farming, and involves thousands of birds grown in complete confinement (battery system or deep litter system). The main characteristics of large scale poultry farms are: use of exotic breeds of birds, use of specially formulated feeds, use of vaccines, and availability of electricity (Kperegbeyi et al., 2009).

Unfortunately, antibiotic use policies are rarely enforced in Nigeria and antibiotics are widely used in poultry farming without oversight (Nsofor et al., 2013; Okonko et al., 2010). Recent surveys (n = 30) indicated that >83% of poultry farmers use tetracyclines, 50% use tylosin, 40% use gentamicin, and <30% use enrofloxacin, ciprofloxacin, penicillin, streptomycin, furazolidone, chloramphenicol, and ampicillin (Nsofor et al., 2013). In the southeastern region of Nigeria, antimicrobial agents were reportedly incorporated directly into poultry feeds, and available without veterinary consult (Nsofor et al., 2013). Among the antibiotics available, tetracycline appears to be the most widely used in African poultry feeds and in some instances as much as 100% of the bacterial isolates from these feeds were resistant to the antibiotics tested (i.e., ampicillin, ampiclox, carbenicillin, ceflaximide, chloramphenicol, ciprocin, ciprofloxacin, cotrimoxazole, erythromycin, floxapen, gentamicin, lincocin, norfloxacin, ofloxacin, penicillin, polymyxin B, reflacine, rifampicin, streptomycin, tetracycline, and vancomycin) (Okonko et al., 2010). These findings are similar to those from other parts of the country such as in northeastern Nigeria (Geidam et al., 2012). These workers observed varying levels of resistances to antibiotics among bacteria isolated from chicken in their studies. This suggests that antibacterial resistances in isolates associated with poultry farms are rampant in Nigeria and portend dangers of treatment failures in cases of infection with such isolates. The demand for poultry is growing in Nigeria, and the resulting poultry litter is used for fertilizing crops and in fisheries.

The purpose of this study was to examine the antibiotic resistance patterns among mesophilic chicken litter bacteria isolated from four different farms in Zaria, northwestern Nigeria. Of particular interest were the distributions of ARGs in the *Escherichia* and *Staphylococcus* genera, which contain several pathogenic members of concern to human health and are increasingly subject to antibiotic treatment failure. Knowledge of the distribution of antibiotic resistant bacteria and ARGs in chicken litter from representative Nigerian chicken farms can provide a point of comparison to countries with varying rearing and antibiotic use practices. Further, identification of potential pathogens that are resistant to important human antibiotics indicates the need for greater attention on ways to prevent and control the spread of antibiotic resistance from livestock operations in developing countries with few antibiotic use restrictions.

MATERIALS AND METHODS

Sample Collection

Four hundred fresh (nonrepeat) poultry fecal droppings were aseptically collected in sterile McCartney bottles from layers in 2 commercial/intensive chicken farms [Department of Animal Science poultry farm, Main Campus, Ahmadu Bello University (i.e., CM) and Accord Farms, Batch C (i.e., C)] and 2 small scale free range farms [Fulani Rarer (i.e., FR) and Area F quarter (i.e., F)] between the hours of 7 and 9 AM over a period of 6 wk (June 4 to July 13, 2012). The farms are located in different parts of Zaria metropolis (Table 1). Samples were immediately transported on ice to the laboratory for analyses (Adeleke and Omafuvbe, 2011; Siwela et al., 2007). A total of 250 samples were randomly selected for the current study.

Isolation and Identification of Bacterial Strains

Ten grams of fecal droppings were homogenized with 90 mL maximum recovery diluents (**MRD**, Oxoid) to obtain 1:10 dilution. Serial dilutions were prepared up to 10^{-5}. A volume of 0.1 mL of the 10^{-3}, 10^{-4}, and 10^{-5} dilutions were plated out on the following agar media for maximum recovery of aerobic mesophilic bacteria: mannitol salt agar-CM0085, Oxoid (Hampshire, United Kingdom) (*Staphylococcus* and related organisms), bile-aesculin agar-CM0888, Oxoid (*Enterococcus*), MacConkey agar-CM 0115, Oxoid and *Salmonella–Shigella* agar-CM0099, Oxoid (*Enterobacteriaceae*). The agar plates were incubated at 37°C for up to 24 h. Representative colonies were selected based on their morphological characteristics and purified on nutrient agar. Tentative biochemical identification of isolates was carried out based on standard

Table 1. Information about the 4 farms from where samples were collected.

Farm identifier	Antibiotic used	Feed	Breed of bird	Life stage	Age of farm	Farming practice description
CM[1]	Oxytetracycline, tylosin, neomycin, chloramphenicol	Self-compounding OR Hybreed, Vitalfeed, Animal Care, Sovetfeed	Isa brown, Black harco	15 mo	>30 yr	Intensive, deep litter farming system
C[2]	Gentamicin, doxycycline, chloramphenicol, tylosin, neomycin, amoxycillin	Self-compounding OR vitafeed, Robson feed, Hybreed	Nova Nera (brown)	14 mo	12 yr	Intensive, deep litter farming system
FR[3]	None	Local grain chaffs	Local breeds[5]	ND	ND	Extensive, local scavenging, supplemented with local grain chaffs
F[4]	None	Local grain chaffs	Local breeds[5]	ND	ND	Extensive, local scavenging, supplemented with local grain chaffs

[1]CM = Department of Animal Science Poultry Farm, Ahmadu Bello University, Zaria, Nigeria.
[2]C = Accord Farms, Kwangila, Zaria (Batch C birds).
[3]FR = Fulani Rearer, Hayin Dogo, Zaria.
[4]F = Area F (household farm), Zaria.
[5]Birds likely coming in contact with commercial feed wastes/antibiotics while roaming.

procedures (Cheesbrough, 2006). Isolates were identified by sequencing the 16S rRNA gene, as described below.

Antibiotic Susceptibility Studies

The agar disc diffusion method as recommended by CLSI (2006) was employed. In all, 17 antibiotics were selected and screened for their inhibitory effects on the isolates based on the procedures of CLSI (2006) and as reported by other workers (Olonitola et al., 2007). *Escherichia* isolates were tested for resistance to amoxicillin/clavulanic acid (**Aug**), ampicillin (**Amp**), cefotaxime (**Cef**), cephalothin (**Cep**), ciprofloxacin (**Cip**), gentamicin (**Gen**), nitrofurantoin (**Nit**), and trimethoprim/sulphamethoxazole (**Cot**). *Staphylococcus* isolates were tested for resistance to Cip, clindamycin (**Cli**), erythromycin (**Ery**), Gen, linzolid (**Lin**), oxacillin (**Oxa**), penicillin (**Pen**), quinopristin/dalfopristin (**Qui**), rifampicin (**Rif**), tetracycline (**Tet**), Cot, and vancomycin (**Van**).

Multiple Antibiotics Resistance Indexing of Isolates

Multiple antibiotic resistance indexing (**MARI**) was based on the procedures of Krumperman (1983). MARI is defined as a/b where "a" represents the number of antibiotics to which an isolate is resistant and "b" the number of antibiotics against which the isolate is tested. An MARI $\geq$ 0.2 is regarded to have originated from environments where antibiotics are frequently used.

Molecular Studies

Individual colonies, in duplicate, were suspended in molecular biology grade water (10 μL), boiled for 10 min, and this solution was used directly for PCR. Prior to Sanger sequencing (Virginia Bioinformatics Institute, Blacksburg, VA), samples were amplified using 8F/1492R primers targeting the V3 region of the 16S rRNA gene (Weisburg et al., 1991). Duplicate sequences were aligned and trimmed using DNA Baser (Heracle BioSoft S.R.L., Pitesti, Romania) with settings for low quality sequences. Multiple sequence alignment was performed using ClustalW and maximum likelihood trees were constructed in MEGA5 (Tamura et al., 2011). The Basic Local Alignment Search Tool (**BLAST**) alignment tool (http://www.ncbi.nlm.nih.gov/BLAST/) was used to identify sequences in GenBank with the highest similarity to the isolated bacteria. BLAST classifications were verified using the Ribosomal Database Project (Wang et al., 2007). Sequences are available in European Molecular Biology Laboratory (EMBL) under accession number LK985325-LK985383.

Testing for presence of ARGs was performed as previously described (Pei et al., 2006) in 25 μL reactions with MasterTaq polymerase (5 Prime, Gaithersburg, MD) for *erm*F (Chen et al., 2007), *mec*A (Volkmann et al., 2004), *sul*1 (Pei et al., 2006), *sul*2 (Pei et al., 2006), *tet*(O) (Aminov et al., 2001), *tet*(W) (Aminov et al., 2001), and *van*A (Clark et al., 1993). These genes were selected to represent a range of ARG representing genes related to antibiotics used at some of the test sites (e.g., *tet* genes for tetracycline, *erm*F gene for macrolides, and *mec*A for methicillin) and *van*A given that it is considered an antibiotic of last resort. No template controls were included in each run. Detection of ARGs was confirmed by agarose gel electrophoresis.

Analysis of Similarity (**ANOSIM**) analysis was performed on transformed ARG presence/absence data and antibiotic susceptibility data using Primer 6 statistical software (PrimerE, Ivy Bridge, United Kingdom).

Table 2. Observed resistance rates among isolates to each antibiotic tested.

Antibiotics	Escherichia % resistant, n = 22	Staphylococcus % resistant, n = 15
Amoxicillin/clavulanic acid (Aug)	0	
Ampicillin (Amp)	22.7	
Cefotaxime (Cef)	0	
Cephalothin (Cep)	13.6	
Ciprofloxacin (Cip)	18.2	0
Clindamycin (Cli)		60.0
Erythromycin (Ery)		80.0
Gentamicin (Gen)	13.6	0
Linzolid (Lin)		13.3
Nitrofurantoin (Nit)	4.5	
Oxacillin (Oxa)		13.3
Penicillin (Pen)		33.3
Quinopristin/dalfopristin (Qui)		26.7
Rifampicin (Rif)		13.3
Tetracycline (Tet)		86.7
Trimethoprim/sulphamethoxazole (Cot)	54.5	0
Vancomycin (Van)		13.3

RESULTS

Identification of Culturable Bacteria and Their Antibiotic Resistance Patterns

Culturable aerobic mesophilic bacteria in the chicken droppings were found to be dominated by *Escherichia* (29.47%), *Staphylococcus* (15.79%), and *Proteus* (15.79%), based on 16S rRNA gene sequencing of a representative subset of isolates (95 out of 250). Other common isolates (>9%) were highest in sequence similarity to *Alcaligenes*, *Pseudomonas*, *Salmonella*, *Enterobacter*, *Klebsiella*, and *Bacillus*. Some of the less commonly encountered genera included *Consenzaea*, *Providencia*, *Lysinibacillus*, and *Wohlfartiimonas*.

A broad range of antimicrobial resistance patterns were observed among the isolates (Tables 2–4). *Escherichia* were uniformly susceptible to augmentin and cefotaxime, while *Staphylococcus* were uniformly susceptible to ciprofloxacin, gentamicin, and trimethoprim/sulfamethoxazole. Over eighty percent of *Staphylococcus* isolates were resistant to tetracycline (Table 2). Many of the isolates were characterized by a MARI $\geq$ 0.2 (Figure 1). Multiple antibiotic resistance was extreme with one particular *Staphylococcus* isolate, which was resistant to 9 antibiotics (Table 4). Among the ARGs tested, the most commonly identified were *sul*1, *sul*2, and *van*A (Tables 3 and 4).

Comparison of Antibiotic Resistance Patterns across Farms

ANOSIM analysis of disc and ARG resistance suites indicated that there was a difference in antibiotic resistance patterns between CM and F ($P = 0.001$) and CM and FR ($P = 0.012$) sample sites for *Escherichia*. In contrast, no differences in resistance patterns were observed between C and either F ($P = 0.30$) or FR ($P = 0.35$) sites for *Escherichia*. There was a difference ($P = 0.022$) between commercial farm CM and free range farm FR for the *Staphylococcus* isolates.

Staphylococcus isolates from free range farms were resistant to zero or 3 antibiotics, while isolates from

Table 3. *Escherichia* resistance patterns for **A)** ARG and **B)** susceptibility tests.

A	Number of ARGs present	Number of isolates with pattern	Resistance pattern (number isolates exhibiting pattern)
	0	5	None
	1	11	*sul*1 (1); *sul*2 (5); *van*A (5)
	2	7	*sul*1/*sul*2 (1); *sul*1/*van*A (2); *sul*2/*van*A (4)
	3	4	*sul*1/*sul*2/*van*A (4)
B	Number of antibiotics	Number of isolates with pattern	Resistance pattern (number isolates exhibiting pattern)
	0	7	None
	1	7	Amp[1] (1), Cep[2] (1), Cot[3] (4), Nit[4] (1)
	2	6	Amp/Cot (3); Gen[5]/Cot (1); Cip[6]/Cot (2)
	4	1	Cep/Cip/Gen/Cot
	5	1	Amp/Cep/Cip/Gen/Cot

[1]Amp = Ampicillin.
[2]Cep = Cephalothin.
[3]Cot = Trimethoprim/sulphamethoxazole.
[4]Nit = Nitrofurantoin.
[5]Gen = Gentamicin.
[6]Cip = Ciprofloxacin.

Table 4. *Staphylococcus* resistance patterns for **A)** ARG and **B)** susceptibility tests.

A	Number of ARGs present	Number of isolates with pattern	Resistance pattern (number isolates exhibiting pattern)
	0	6	None
	1	2	*sul*2 (1); *van*A (1)
	2	2	*sul*1/*van*A (1); *sul*2/*van*A (1)
B	Number of antibiotics	Number of isolates with pattern	Resistance pattern (number isolates exhibiting pattern)
	0	1	None
	1	1	Tet[1]
	2	2	Tet/Ery[2]
	3	7	Oxa[3]/Pen[4]/Rif[5] (1); Ery/Qui[6]/Tet (1); Cli[7]/Ery/Tet (5)
	4	1	Cli/Ery/Pen/Tet
	5	1	Cli/Ery/Pen/Qui/Tet
	7	1	Cli/Ery/Lin[8]/Pen/Qui/Tet/Van[9]
	9	1	Cli/Ery/Lin/Oxa/Pen/Rif/Qui/Tet/Van

[1]Tet = Tetracycline.
[2]Ery = Erythromycin.
[3]Oxa = Oxacillin.
[4]Pen = Penicillin.
[5]Rif = Rifampicin.
[6]Qui = Quinopristin/dalfopristin.
[7]Cli = Clindamycin.
[8]Lin = Linzolid.
[9]Van = Vancomycin.

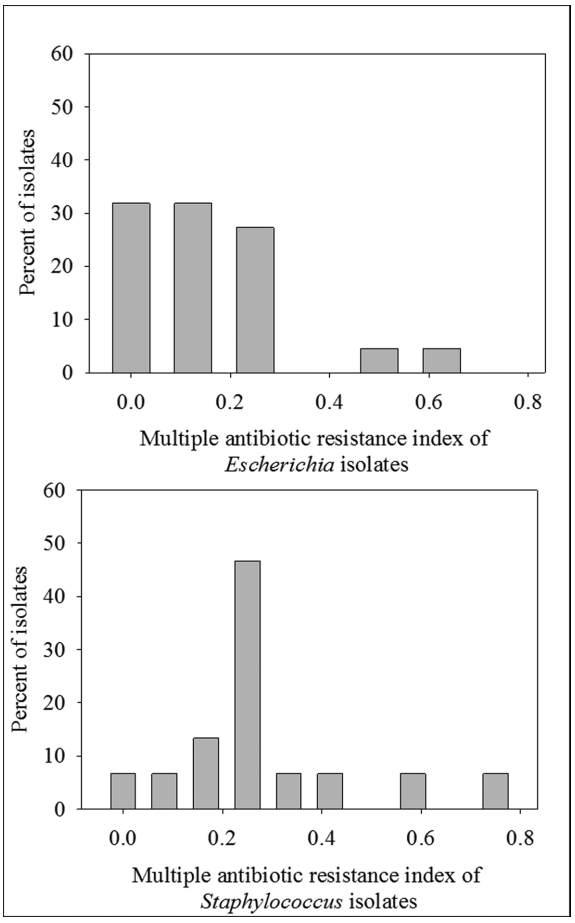

Figure 1. Multiple antibiotic resistance index (MARI) for **A)** *Escherichia* (N = 22), **B)** *Staphylococcus* isolates (N = 15).

the commercial/intensive farms (Department of Animal Science Farm and Accord Farms) were resistant to one to 7 antibiotics. No resistance was the most commonly observed pattern for isolates from the free range farms (Fulani Rearer Farm and Area F Farm); however the isolates with the highest MARI, those resistant to 4 of 5 antibiotics, were also observed from the free range farms. The most commonly observed resistance pattern for isolates from the commercial farms was to one antibiotic (n = 6).

Escherichia isolated from free range farms (9 isolates total) were resistant to zero up to 5 antibiotics, with no resistance the most commonly observed pattern (4 isolates). *Escherichia* isolated from commercial farms (13 isolates) were resistant to zero to 3 antibiotics with resistance to one antibiotic the most commonly observed (6 isolates). Therefore, while *Escherichia* isolates with the highest MARI were found on free range farms, a higher incidence of resistance (10/13 on commercial vs. 5/9) was observed for isolates from commercial farms.

Of the 7 ARGs investigated in this study (*erm*F, *mec*A, *sul*1, *sul*2, *tet*O, *tet*W, and *van*A) only *sul*1, *sul*2, and *van*A were detected. For *Escherichia, sul*1, *sul*2, and *van*A were detected in isolates on each type of farm. Most isolates from the Area F farm/Fulani Rearer farm (7/15) contained one ARG, for the Department of Animal Science Farm and Accord Farms 4 isolates contained one ARG and 4 contained 2 ARGs. ARGs were detected in neither of 2 *Staphylococcus* isolates from the Area F farm/Fulani Rearer farm, while resistance patterns of zero, one, and 2 ARGs were each observed in 4 isolates from the Accord Farms/Department of Animal Science farm. For *Staphylococcus*, one strain was vancomycin resistant and tested positive for the *van*A gene, 2 strains had the gene but were susceptible to vancomycin, and one strain displayed resistance to vancomycin but the *van*A gene was not detected. Of the 22 *Escherichia* isolates tested for both susceptibility and ARGs, 8 were resistant to Cot and contained the *sul*2 gene, 4 were resistant to Cot and contained both *sul*1 and *sul*2 genes, while the rest demonstrated no resistance to Cot and either contained the *sul*1 and *sul*2 genes (one isolate), contained *sul*2 gene (3 isolates), or contained neither *sul* gene tested (6 isolates). In contrast, no resistance to Cot was observed in *Staphylococcus* isolates while 2 isolates tested positive for the *sul*2 gene and one carried the *sul*1 gene.

DISCUSSION

This study provides insight into the status of antimicrobial resistance in poultry litter in northwestern Nigeria. This part of the world is of particular interest given that antimicrobials are widely used in livestock and poorly controlled and that little data is available on the effects of varying rearing practices. Further, human populations in Africa are susceptible to relatively high rates of antibiotic resistance bacterial infection (Pablos-Méndez et al., 1998). Thus, a larger concern is not just the presence of potentially pathogenic bacteria resistant to antibiotics in poultry litter, but their potential to spread through the environment and to humans, given the local farming practices. Others have observed, in both developing and industrialized nations, that a wide range of ARGs from agricultural operations can disseminate and persist in affected environments (Pei et al., 2006). In particular, high levels of ARGs have been measured in poultry litter from intensive poultry facilities (Diarrassouba et al., 2007).

Association of Genera Isolated with Human Pathogens

Of particular concern is that many of bacterial genera isolated are associated with important human pathogens. The identification of the isolates obtained were generally consistent with a previous report from Ile-Ife, southwestern Nigeria (Adeleke and Omafuvbe, 2011), though a few differences were noted. In particular, *Corynebacterium*, *Acinetobacter*, and *Aeromonas* were reported in the Ile-Ife study and not found in the present study, while *Providentcia*, *Cocenzaea*, *Lysinibacillus*, *Wolhfahrtiimonas*, *Klebsiella*, and *Enterobacter* were encountered in this study, but not the

Ile-Ife study. These discrepancies could be affected by the geographical differences (Ile-Ife is in southwestern Nigeria) (Ojo et al., 2012) or differences in isolation techniques. Nonetheless, most of the universally reported organisms in poultry fecal samples, including *Escherichia, Shigella, Staphylococcus, Proteus, Salmonella*, and *Bacillus* were isolated in both studies. In addition, the 2 studies encountered higher numbers of Gram negative than Gram positive bacteria, as might be expected given these bacteria are normal flora of the intestinal tract of poultry birds (Hemen et al., 2012). Interestingly, some of the bacteria encountered, including *Bacillus*, *Escherichia*, *Staphylococcus*, *Pseudomonas*, and *Salmonella* have been isolated liberally from commercial chicken feeds in Calabar, the southernmost geopolitical zone of Nigeria (Okonko et al., 2010), suggesting that potentially pathogenic antibiotic resistant bacteria could easily be circulated via feeding operations.

Observed Patterns of Antibiotic Resistance and Implications

This study confirms that resistant strains of several bacteria are circulating in Nigerian poultry operations, as reported in other studies (Nsofor et al., 2013). *Escherichia* displayed a high rate of resistance to trimethoprim/sulfamethoxazole (tested on same disc) (54.5%), ampicillin (22.7%), ciprofloxacin (18.2%), cephalothin (13.6%), and gentamicin (13.6%). However, the resistance rates among isolates in the present study appear to be generally lower than some of those previously reported cases in Nigeria, which ranged from 60 to 100% resistances in most instances (Geidam et al., 2012; Ojo et al., 2012). The lower percentage resistance observed in the current study may be due to the fact that laying hens were studied, rather than broilers. van den Bogaard et al. (2001) reported that bacterial infections occur less frequently in layers than in young broilers and consequently less antibiotics tend to be used.

While a high level of indiscriminate use of antimicrobials in animals has been reported, due to weak enforcement of antibiotic use policies (Ojo et al., 2012), findings of the current study suggest that overuse of antibiotics may not be prominent on medium/small scale poultry farms in northwestern Nigeria. This may be in part because use of antibiotics in laying birds presents additional cost to farmers that may not present benefits for outputs of laying hens. Still, the rate of *Escherichia* resistances observed in the present study agree with those reported in Australia (Obeng et al., 2012), where use of antimicrobials in animal production is regulated and enforced. Studies of intensive chicken farms in north east Georgia, United States indicated that 75% of *E. coli* isolates from chicken litter were resistant to 3 or more antibiotics (Smith et al., 2007), whereas the present study noted 91% of isolates to be resistant to 2 or fewer antibiotics. This is consistent with observations of lower rates of antibiotic resistance on organic versus commercial poultry farms in the United States (Sapkota et al., 2011).

The observed resistance rate to the fluoroquinolone antibiotic, ciprofloxacin (18.2%), is comparable to the 16.7% reported by Ojo et al. (2012), but is in contrast to 0% resistance reported by some researchers (Olaitan et al., 2011). Flouroquinolones are not used in animal production in Nigeria, due to the high costs, but possible exposure to less potent and adulterated antibiotics by humans sharing the same environments with the farm animals may be responsible for the possible emergence of resistance to these less misused antibiotics (Nsofor and Iroegbu, 2012). This study indicates that even resistance to important human antibiotics not used in livestock is possible when such antibiotics are not being used and the need for comprehensive control strategies. ANOSIM analysis indicated that patterns of resistance were not necessarily strongly associated with sample origin and therefore known antibiotic use; however, it is expected given a larger sample size such patterns may become apparent given a larger sample size.

In this study, resistances to the tested antibiotics were even higher in *Staphylococcus* than in *Escherichia*. These findings are in agreement with Suleiman *et al.* (2013) and further support the fact that antimicrobial drug resistance rates among pathogen-containing genera are a concern in Nigeria. This is corroborated by the MARI of the bacterial isolates in the present study. At least 61.5% (*Staphylococcus*) and 33.3% (*Escherichia*) of the isolates were characterized by a MARI ≥ 0.2, which is usually an indication that the isolates originated from environments where antibiotics are frequently used (Krumperman, 1983). It is also interesting to note that 65.4% of the *Staphylococcus* isolates expressed multiple resistances to at least 3 antibiotics, particularly among strains isolated from intensive/commercial farms where antibiotic use is more common. Multiple antibiotic resistances have previously been reported in *Staphylococcus* in Nigeria (Okonko et al., 2010), suggesting that it is probably a common occurrence around the country.

Distribution of Targeted ARGs

Of the 7 ARGs investigated in this study, only *sul*1, *sul*2, and *van*A were detected. The ARG *van*A was the most frequently detected among *Staphylococcus* (30%), while *sul*2 was the most frequently detected in *Escherichia* (77.2%). Still, *van*A was detected in 68.2% of *Escherichia* isolates.

Vancomycin is a narrow spectrum inhibitor of cell wall synthesis in bacteria. Vancomycin is widely used to treat many life-threatening human infections caused by Gram positive bacteria (Sanakal and Kaliwal, 2011). Resistance to vancomycin is mediated by the *van*A gene in *Staphylococcus aureus* (Chang et al., 2003) and it was detected in 3 *Staphylococcus* isolates in the present

study. Detection of the *van*A gene where resistance was not demonstrated may be due to bacteria harboring an inactive or damaged gene that is not longer functional, which is possible given the length of insert the *van*A protocol targets. These results may indicate other genes are involved in vancomycin resistance given that strains displayed resistance without the gene.

Although vancomycin resistance is not indicated for *Escherichia*, because it is a Gram negative bacterium, several isolates carried the *van*A gene. This suggests that carrier strains that do not express resistance could mediate horizontal gene transfer of resistance to critically important antibiotics (Kristiansson et al., 2011).

The genes *sul*1 and *sul*2 were frequently detected in this study and detection of *sul*2 was often associated with resistance to sulfamethoxazole. No isolates demonstrated resistance to sulfamethoxaole without the detection of *sul*1 or *sul*2, but *sul*1 and *sul*2 were also detected in some isolates that did not demonstrate resistance to sulfamethoxaole. This indicates that ARGs may be present, but inactive. Others have noted that sulfonamide resistance is common in bacteria from swine and chickens (Kozak et al., 2009).

The *mec*A gene encodes resistance to methicillin. Although some of the *Staphylococcus* isolates were resistant to oxacillin, none of them carried *mec*A gene. Previous studies in Zaria, Nigeria, also failed to detect the *mec*A gene, even in confirmed Methicillin-resistant Staphylococcus aureus (**MRSA**) (Olayinka et al., 2009; Olonitola et al., 2007), thus it was suggested by Olayinka et al. (2009) that the MRSA phenotype observed in their isolates was not the classical *mec*A-mediated resistance, but most probably due to hyperproduction of β-lactamase.

In the case of tetracyclines, several genes confer resistance by various mechanisms, whereas this study investigated the ribosomal protection factors *tet*(O) and *tet*(W) and did not find them to be present in any of the strains. Others have observed *tet*(A) and *tet*(B) in commercial broiler chicken litter from British Columbia, Canada (Diarrassouba et al., 2007).

The *erm*F gene confers resistance to macrolides, lincosamides, and streptogramin B (Chung et al., 1999). While several of the isolates displayed resistance to erythromycin and clindamycin, the *erm*F gene was not detected in any of the *Staphylococcus* isolates. Thus, another gene may be responsible for the observed resistance or the resistance was phenotypic in nature (Bae et al., 2007).

CONCLUSIONS

Antibiotic resistance patterns were profiled in mesophilic aerobic bacterial isolates obtained from free range and commercial chicken farms in Zaria, northwestern Nigeria, a part of the world with little data to address the effect of farming practices on antibiotic resistance. Of particular concern was that genera containing pathogenic members were the most commonly identified and that many of the antibiotic resistance patterns indicated multiple antibiotic resistance and potential for antibiotic treatment failure. Vancomycin resistance was common among *Staphylococcus* isolates and the *van*A gene was the most frequently detected of the ARGs assayed. Observed resistance rates in *Escherichia* were lower than those observed in litter from commercial chicken operations reported in industrialized nations; however, Nigerian practices may increase the potential for transfer to humans. For example, free range farming practices, may present benefits from high turnover rates and low animal counts per acre, but increase potential for human contact and infection transfer compared to commercial farms. Further study is recommended to demonstrate which farming practices best reduce antibiotic resistance levels and minimize the potential to spread to humans.

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