Research Article

Fecal Antibiotic Resistome of Pigs from a Small-Scale Piggery in Ibadan, South-West Nigeria

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Abstract | This study was designed to sample the fecal Resistome of Pigs from a small-scale Piggery in Ibadan, South-West Nigeria. Three fecal pellets were randomly picked from the floor of unit pens containing a minimum of three pigs per unit in selected piggery in Ibadan, Nigeria. The samples were pooled and resuspended in phosphate buffered saline. The suspension was then subjected to nucleic acid extraction, cDNA synthesis and Illumina sequencing. Antibiotic Resistance Genes (ARGs) in the raw reads were determined and assembled using the Kmer Resistance tool v2.2. From the 2,974,257 reads generated, 21 ARGs with statistically significant reads were identified. Almost all targeted broad-spectrum antibiotics with over 50% targeting Tetracyclines. Five (*ant(6)-Ia_3, tet(40)_1, tet(Q)_1, tet(W)_5 and tet(O/W)_4*) of the ARGs were predicted to be plasmid-borne. Our findings show that the Swine industry in the region might be both a mixing pot and reservoir of ARGs. It is therefore crucial that effort is made to educate the stakeholders on the importance of good antibiotics stewardship.

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Introduction

The core message of the One-Health movement is the fact that human health is on an intricate balance pivoted by the entire health of physical environment and surrounding livestock and game animals (Ryu et al., 2017; World Bank, 2010). This fragile relationship has been well documented in

history, especially of outbreaks in human populations caused by zoonotic pathogen spillover events (Woolhouse and Gaunt, 2007; Daszak et al., 2000; Jones et al., 2008) As humans, our most intimate and economic interactions with animals are livestock, hence, we have a significant amount of documented zoonotic outbreaks originating from livestock to humans (Woolhouse and Gaunt, 2007; Daszak et al., 2000; Jones et al., 2008).

Swine production is a major sector in the livestock industry in Nigeria with over 7.1 million pigs as of 2011 (Igbokwe and Maduka, 2018). Critically, there is paucity of data on population statistics of domestic animals in Nigeria, however, inferential deductions from the proliferation of small scale and peasant pig farming show possibility of increased pig population in Nigeria. Moreover, in recent years, there is a conspicuous renewed interest in swine production, partly, due to their prolificacy, impressive growth rate, relative hardiness and ability to thrive on readily available agro-industrial waste products especially in the face of dwindling disposable income and the increasing population in Nigeria. In south-west Nigeria, many small-scale piggeries exist and contribute significantly to meat production in the region. These facilities have 1 to 50 pigs and on occasion consult with Veterinarians and other Animal Health Experts (AHEs) for health services. However, due to the small-scale nature of these businesses,

Table 1: *Antibiotic resistance genes recovered from pig feces during this study.*

S. No	Antibiotic resistance gene	Score	Expected	Template length	q_value	p_value	Template id	Template coverage	Query id	Query coverage	Depth	Depth corr
1	ant(6)-Ia_3_KF864551	8670	99	867	8376.88	1.00E-26	100	100	100	100	10.01	0.9267
2	ant(6)-Ia_1_AF330699	1311	106	909	1023.7	1.00E-26	71.4	72.28	98.78	138.36	1.55	0.3328
3	ant(6)-Ib_1_FN594949	1212	100	858	941.34	1.00E-26	85.78	85.78	100	116.58	1.41	0.308
4	aph(3')-III_1_M26832	1058	93	795	808.72	1.00E-26	70.06	71.82	97.55	139.23	1.45	0.3152
5	aph(3'')-Ib_5_AF321551	1056	94	804	804.27	1.00E-26	69.4	69.78	99.47	143.32	1.35	0.2971
6	blaACI-1_1_AJ007350	1403	100	855	1129.45	1.00E-26	92.28	94.27	97.89	106.08	1.74	0.3651
7	erm(B)_1_JN899585	2306	86	738	2060.01	1.00E-26	92.01	92.01	100	108.69	3.13	0.5583
8	mef(A)_3_AF227520	6135	140	1218	5726.1	1.00E-26	80.79	84.56	95.53	118.25	5.98	0.7901
9	lnu(C)_1_AY928180	1915	57	495	1748.28	1.00E-26	88.28	89.49	98.65	111.74	4.07	0.6545
10	sul2_1_AF542061	870	95	816	620.93	1.00E-26	61.52	61.64	99.8	162.23	1.08	0.2457
11	tet(44)_1_NZ_ABDU01000081	3419	223	1923	2802.72	1.00E-26	66.46	67.76	98.08	147.58	1.89	0.3895
12	tet(44)_2_FN594949	5962	221	1923	5327.9	1.00E-26	84.87	85.86	98.85	116.47	3.24	0.5709
13	tet(40)_1_FJ158002	14470	137	1221	14063.63	1.00E-26	99.84	100	99.84	100	12.09	0.9574
14	tet(Q)_1_L33696	137368	131	1926	136975.1	1.00E-26	99.95	100	99.95	100	72.88	1
15	tet(Q)_2_X58717	6447	222	1926	5810.56	1.00E-26	68.59	70.61	97.13	141.62	3.59	0.6083
16	tet(Q)_4_Z21523	10658	219	1926	10018.42	1.00E-26	90.08	91.85	98.08	108.88	5.83	0.7818
17	tet(W)_2_AY049983	5619	221	1920	4987.07	1.00E-26	93.18	95.83	97.23	104.35	3.09	0.5537
18	tet(W)_5_AJ427422	40577	197	1920	39987.76	1.00E-26	99.95	100	99.95	100	21.66	0.9965
19	tet(O/W)_4_AM889121	19724	208	1889	19106.66	1.00E-26	70.67	70.67	100	141.5	10.65	0.938
20	tet(O/W/O)-2_1_AY196920	10118	218	1920	9480.22	1.00E-26	79.79	80.16	99.55	124.76	5.4	0.7558
21	tet(O/32/O)_7_FP929050	8174	220	1920	7536.75	1.00E-26	64.27	64.64	99.44	154.71	4.38	0.6813

Note: *Antibiotic Resistance Gene: shows the name of the template sequences; Score: is the global alignment score of the template; Expected: is the expected alignment score if all mapping reads where smeared over all templates in the database; Template length: is the template length in nucleotides; q_value: is the quantile in a standard Pearson Chi-square test, to test whether the current template is a significant hit; p_value: is p-value corresponding to the obtained q-value.; Template_id is the percent identity of the found template, over the full template length; Template_coverage is percent of the template that is covered by the query; Query_id is the percent identity between the query and template sequence, over the length of the matching query sequence; Query_coverage is the length of the matching query sequence divided by the template length; Depth: is the number of times the template has been covered by the query; Depth_Corr: is an Estimate of how good the depth of the current template is compared to the found host, a low value would point towards contamination, a value around 0.5 would indicate that the gene is located on the host genome and a value close to 1.0 would indicate that this template is plasmid borne.*

the managers/farmers tend to quack (Omotosho et al., 2013, 2016) and self-medicate (based on their experience from previous consultations with AHEs) the animals without consultation with Veterinarians and other AHEs, a practice that has resulted into indiscriminate use, low dosage use in feed and out of prescription use of antimicrobials (Van et al., 2020).

These practices are certain to result in the development of antibiotic resistant bacteria in pigs in the region. Antibiotic resistance (AR) in different bacterial types recovered from Pigs in the region have been documented (Oloso et al., 2018). These studies reviewed with reductionist approach were designed around result in a bacteria by bacterial AR profile and do not give a global view of the constellation of AR genes (ARGs) (called the Resistome) in the bacteriome (Microbiome) of Pigs in the region. Hence, in this study we attempt to sample the Resistome of the fecal Bacteriome of Pigs from a small scale Piggery in Ibadan, south-west Nigeria.

Materials and Methods

Sample collection

Fecal samples of pigs were analysed in this study. The fecal samples were collected in June 2018 from a Pig farm in Ibadan, Oyo State, south-west Nigeria. The sample is a pool made from fecal pellets on the floor of a Pen containing three Pigs. Three independent randomly selected fecal pellets were selected. Each pellet was broken and about 3grams from the core was inserted in a 15mL centrifuge tube. All three pellets were collected into the same sample tube.

Processing for illumina sequencing

The fecal pool was resuspended in phosphate buffered saline (PBS). Afterward, nucleic acid was extracted from the suspension using the DNA/RNA extraction kit (Jena Biosceince, Jena, Germany). Subsequently, cDNA was synthesized using the SCRIPT cDNA synthesis kit (Jena Biosceince, Jena, Germany). This was then shipped to a commercial facility (MR DNA, Texas, USA) where library preparation and sequencing was done. The library was prepared using the TruSeq™ RNA LT Sample Preparation Kit (Illumina) as recommended by the manufacturer. Subsequently, sequencing was done paired end for 300 cycles using the HiSeq system (Illumina).

Bioinformatic analysis

The quality of the raw reads was assessed using the FASTQC tool v1.0.4. Subsequently, the ARGs in the raw reads were determined and assembled using the Kmer Resistance tool v2.2 (https://cge.cbs.dtu.dk/services/KmerResistance/) with default parameters (Clausen et al., 2018) The detected and assembled ARGs were then downloaded, visually screened and those for which single contigs exceeded 200bp were further analyzed and submitted to GenBank under the accession numbers MK286928, MK293762-MK293776.

Results and Discussion

From the 2,974,257 reads generated, 21 ARGs with statistically significant reads were identified (Table 1). Almost all the ARGs detected targeted broad-spectrum antibiotics and fell into three modes of action (Table 2). Precisely, 90.5% (19/21) of the ARGs detected target drugs that inhibit translation. The remaining 9.5% (2/21) inhibit cell wall synthesis (*blaACI*) and nucleic acid synthesis by disruption of single-carbon metabolism (*sul2*). Estimates of how good the depth of the current template is compared to that found in host (Table 1; Depth corr >0.8) suggest that some (*ant(6)-Ia_3, tet(40)_1, tet(Q)_1, tet(W)_5 and tet(O/W)_4*) of the ARGs detected are likely to be plasmid borne. The complete coding sequence (CDS) of four of the five (5) genes that seem to be plasmid borne were recovered.

Diversity of ARGs detected and what it implies for antibiotic use

In this study we investigated the Resistome of Pigs farmed on a small scale piggery in Ibadan, south-west, Nigeria in an effort to appraise what it will reveal about antibiotic use in Piggery in the region. Particularly, we sequenced cDNA. Hence, might be assessing genes that were expressed in the fecal microbiome of the Pigs. In all, we detected 21 ARGs (Table 1) which target drugs that inhibit translation, cell wall synthesis and nucleic acid synthesis (Table 2). While antibiotics that inhibit translation and nucleic acid synthesis are broad-spectrum, the current generation of antibiotics that inhibit cell wall synthesis could also be broad-spectrum. The results of this study therefore show that almost all the ARGs detected, target broad-spectrum antibiotics. This suggests a possible ongoing treatment of the animals in the farm and dependence on broads spectrum antibiotics in Swine management in the region.

Table 2: *Classification and mechanism of action of the antibiotic resistance genes detected during this study.*

S. No	Mode of action	Antibiotic resistance gene	Class of target drug	Cheapest brand available locally and commonly used in piggery	Resistance mechanism
1	Inhibition of translation	ant(6)-Ia_3	aminoglycoside	Gentamycin and Amikacin	Aminoglycoside adenyltransferases
2	Inhibition of translation	ant(6)-Ia_1	aminoglycoside	Gentamycin and Amikacin	Aminoglycoside adenyltransferases
3	Inhibition of translation	ant(6)-Ib_1	aminoglycoside	Gentamycin and Amikacin	Aminoglycoside adenyltransferases
4	Inhibition of translation	aph(3')-III_1	aminoglycoside	Gentamycin and Amikacin	Aminoglycoside phosphotransferases
5	Inhibition of translation	aph(3'')-Ib_5	aminoglycoside	Gentamycin and Amikacin	Aminoglycoside phosphotransferases
6	Inhibition of translation	erm(B)_1	macrolide	Erythromycin and Tylosin	Erm 23S rRNA methyltransferases
7	Inhibition of translation	mef(A)_3	macrolide	Erythromycin and Tylosin	Macrolide resistance efflux pumps
8	Inhibition of translation	lnu(C)_1	lincosamide	Lincomycin and Clindamycin	Lincosamide nucleotidyltransferase (Lin)
9	Inhibition of translation	tet(44)_1_NZ	Tetracycline	Oxytetracycline 20%	Tetracycline resistance ribosomal protection proteins
10	Inhibition of translation	tet(44)_2	Tetracycline	Oxytetracycline 20%	Tetracycline resistance ribosomal protection proteins
11	Inhibition of translation	tet(40)_1	Tetracycline	Oxytetracycline 20%	Tetracycline resistance ribosomal protection proteins
12	Inhibition of translation	tet(Q)_1	Tetracycline	Oxytetracycline 20%	Tetracycline resistance ribosomal protection proteins
13	Inhibition of translation	tet(Q)_2	Tetracycline	Oxytetracycline 20%	Tetracycline resistance ribosomal protection proteins
14	Inhibition of translation	tet(Q)_4	Tetracycline	Oxytetracycline 20%	Tetracycline resistance ribosomal protection proteins
15	Inhibition of translation	tet(W)_2	Tetracycline	Oxytetracycline 20%	Tetracycline resistance ribosomal protection proteins
16	Inhibition of translation	tet(W)_5	Tetracycline	Oxytetracycline 20%	Tetracycline resistance ribosomal protection proteins
17	Inhibition of translation	tet(O/W)_4	Tetracycline	Oxytetracycline 20%	Tetracycline resistance ribosomal protection proteins
18	Inhibition of translation	tet (O/W/O)-2_1	Tetracycline	Oxytetracycline 20%	Tetracycline resistance ribosomal protection proteins
19	Inhibition of translation	tet (O/32/O)_7	Tetracycline	Oxytetracycline 20%	Tetracycline resistance ribosomal protection proteins
20	Inhibition of cell wall synthesis or disruption of membrane	blaACI-1_1	beta-Lactamase		Class A β-lactamases
21	Inhibition of nucleic acid synthesis by disruption of single-carbon metabolism	sul2_1_	sulfonamide	Sulfafurazole and Sulfasomidine	Sulfonamide-resistant dihydropteroate synthases

Considering that farm managers tend to self-medicate the pigs based on previous consultations with AHEs, it is likely that AHEs in the region use broad-spectrum antibiotics significantly. This could be due to economic reasons. Specifically, most of the small-scale Pig farm managers are not (even if recommended) willing to pay for laboratory diagnosis of any clinical condition in their few farm animals. Rather, sick animals are sold to slaughter houses or slaughtered on the farm for consumption. Consequently, AHEs treat the Pigs based on symptoms and recommend broad spectrum antibiotics to ensure that the infection is controlled, irrespective of the etiological agent. The consequence of this practice is the accumulation of resistance to broad spectrum antibiotics in the intestinal/fecal bacteriome of Pigs in the region; as documented in this study.

It should be noted that over 50% (Tables 1 and 2) of the ARGs detected in this study are targeted at Tetracyclines. This is not surprising because, in addition to using Tetracyclines for treatment (or prevention) of infections, Tetracyclines like chlortetracycline and oxytetracycline are used at suboptimal levels for their animal growth-promoting properties (Gustafson and Kiser, 1985; Aerestrup, 2015) This allows the emergence of AR bacteria by giving selective advantage to strains with ARGs specific for the administered antibiotic. Hence, the combination of using Tetracyclines for both growth promotion and treatment, encourages emergence of Tetracycline resistance genes and subsequently selects for it in the Pig microbiome.

Plasmid encoded ARGS and horizontal gene transfer

The results of this study showed that some (*ant(6)-Ia_3, tet(40)_1, tet(Q)_1, tet(W)_5 and tet(O/W)_4*) of the ARGs detected are likely to be Plasmid borne. Our findings in this respect conform with what is documented in literature (Mendez et al., 1980; Jones et al., 1992; Recchia and Hall, 1995; Chopra and Robert, 2001). Being plasmid borne, these ARGs targeted at broad-spectrum antibiotics are transferred horizontally and consequently, spread among and between bacterial species. Ryu et al. (2017) Considering zoonotic spread of pathogens from Pigs to Humans, it is not surprising that these plasmid-borne ARGs (targeting broad spectrum antibiotics) are present in the intestinal microbiome of humans (Chopra and Robert, 2001). We however posit that they are likely to be present at higher rates in livestock handlers in the region. This has serious health implications for the people working in the Swine and allied industries. It is therefore important that the prevalence of ARGs targeted at broad spectrum antibiotics in this population be determined as it might directly impact the treatment of bacterial infections in these population.

In summary, we sampled the Resistome of Pigs farmed on a small-scale piggery in Ibadan, southwest, Nigeria and found 21 ARGs that target broad spectrum antibiotics that inhibit translation, cell wall synthesis and nucleic acid synthesis. Our data also suggest that some of these ARGs might be plasmid borne and consequently involved in horizontal gene transfer. Finally, our findings show that the Swine industry in the region might be both a mixing pot and reservoir of ARGs. It is therefore crucial that effort is made to educate the stake-holders on the importance of good antibiotics stewardship.

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Novelty Statement

This study particularly is the first of its kind to focus primarily on Antibiotic Resistance Genes (ARGs)/ Antibiotic Resistome from faecal materials of domestic pigs (Sus scrofa domesticus) in farm settlements of pig businesses in Ibadan. The study reports 21 ARGs with global scores and especially for the first time reporting the ARG; Lnu(C)_1 from the Drug Class Lincosamide, a broad-spectrum antibiotic used in Ibadan city, Southwest Nigeria.

Author's Contribution

TOCF, OMA and JAA conceptualized the research. All authors were involved in sample collection and laboratory preparation of samples. TOCF analyzed the data and interpreted the results. OMA and JAA supervised the work. All authors were responsible for writing and reviewing the final manuscript.

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Conflict of interest

The authors have declared no conflict of interest.

Ethics approval

Not required. Sampling was not invasive and was precisely done without contact with the Pigs.

Availability of data and material

Sequence data generated from the study have been submitted to GenBank under the accession numbers MK286928, MK293762-MK293776

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