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Whole-Genome Characterization of Gram-Negative Bacteria in Pig Production Systems at Okada, Edo State, Nigeria: A One Health Approach

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Abstract
Pig farming significantly supports livestock agriculture, food security, livelihoods, household income, and employment across developing countries, including Nigeria. This study characterized Gram-negative bacteria from pig production systems at Okada, Edo State, Nigeria, using a One Health approach of integrating animal, environmental and human-associated compartments. A total of 356 samples were collected and examined from the three compartments comprising, 132 pig-associated (37.08%), 124 environmental (34.83%), and 100 human-associated (28.09%). Cultural and biochemical examinations yielded 48 Gram-negative isolates, with 22 (45.83%) from pigs, 14 (29.17%) from environmental sources, and 12 (25.00%) from humans. The isolates comprised Proteus, Enterobacter, Pseudomonas, Escherichia, Klebsiella, Bordetella, Morganella, Providencia *and* Citrobacter, *with* Proteus *predominating (13; 27.08%). Although bacterial genera varied numerically among compartments, Pearson's chi-square test showed no significant association between bacterial genus and compartment ($\chi^2 = 11.25$, $p = 0.79$). Whole-genome shotgun sequencing generated high-quality data, with mean reads exceeding 17 million and Q30 scores, with 95.50–96.50% of reads retained after filtering. Krona analysis showed predominance of Proteobacteria, Gammaproteobacteria and Enterobacterales across the compartments. Proteus spp (> 20%) and* Bordetella trematum*(16%), were prominent among pig-associated sequences; Proteus spp (>22%) and* Escherichia coli*(14%), predominated among human-associated sequences; environmental profiles were more heterogeneous, with* E. coli, Citrobacter freundii, Klebsiella variicola *each contributing approximately 9%. Seventeen species and a genus Halomonas were identified, with nine species shared across the three compartments. Cochran's Q test showed no significant difference in species representation ($Q = 1.14$, $p = 0.57$). Jaccard similarity was highest between environmental and human-associated compartments (0.733), indicating substantial taxonomic connectivity. Overall, the findings support integrated One Health surveillance of Gram-negative bacteria in pig production.*

Keywords: *Gram-negative bacteria; Whole-genome sequencing; One Health; Pig production system; Taxonomic connectivity*

1. Introduction

Pig production is an important component of livestock agriculture and contributes substantially to food security, household income and employment in many developing countries, including Nigeria. As pig farming becomes increasingly important for meeting the demand for animal protein, concerns about bacterial pathogens and antimicrobial resistance within production systems have also increased. Pig farms provide an ecological interface where animals, farm workers, feed, water, soil, manure and wastewater interact continuously. Consequently, microorganisms circulating within these systems may move between animals and humans or become established in surrounding environmental compartments. The interconnected nature of these pathways makes livestock production an important area for investigation from a One Health perspective [1, 2]. Gram-negative bacteria are particularly important in this context because many species occurring in livestock environments are either established pathogens or opportunistic organisms capable of causing infections in humans and animals. Members of the families Enterobacteriaceae and related groups, including *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp. and *Salmonella* spp., may occur in the gastrointestinal tract of pigs, animal waste, contaminated water, soil and farm surfaces. Some strains possess virulence-associated characteristics that enable them to cause intestinal, urinary, systemic and other infections. More importantly, these organisms can acquire and disseminate antimicrobial resistance (AMR) determinants, thereby creating reservoirs of resistance within food-animal production systems [1, 3]. Antimicrobial resistance has become a major global health concern because infections caused by resistant organisms are increasingly difficult to treat. Although antimicrobial drugs remain essential for the prevention and treatment of bacterial infections, their inappropriate or excessive use creates selective pressure that favours resistant populations. This concern extends beyond human medicine because antimicrobial agents are also used in livestock production for therapeutic, metaphylactic and, in some settings, prophylactic purposes. Resistant bacteria selected within animals can subsequently enter the environment through faeces and manure and may be disseminated through water, soil, farm workers, food products and animal movement [1, 2]. The World Health Organization [4] therefore recognizes AMR as a problem that requires coordinated action across human, animal and environmental health sectors. The environmental component of pig production is particularly relevant because manure and wastewater may contain large populations of bacteria together with antimicrobial residues and resistance genes. When animal waste is inadequately treated or disposed of, these materials can contaminate agricultural soils and surface or groundwater, creating opportunities for resistant organisms and resistance determinants to persist and spread. Woolhouse [2] described this process as an interconnected AMR ecosystem in which humans, animals and the environment are linked through direct contact, food chains, manure application, wastewater and pollution. Similar concerns have been emphasized in developing countries, where limitations in antimicrobial stewardship, environmental sanitation and surveillance may increase the likelihood of resistance dissemination [3]. Nigeria faces particular challenges in addressing AMR because antimicrobial use occurs across human health, veterinary medicine, agriculture and food-production systems. A recent review of AMR in Nigeria identified inappropriate antimicrobial use, inadequate stewardship, environmental contamination and insufficient surveillance

as important factors sustaining the problem and emphasized the need for integrated One Health interventions [5]. In response to these challenges, Nigeria developed its Second One Health Antimicrobial Resistance National Action Plan 2024–2028, emphasizing multisectoral surveillance, antimicrobial stewardship and coordinated action involving human, animal and environmental health sectors [1, 6]. These national priorities demonstrate the increasing recognition that AMR surveillance should extend beyond hospitals to agricultural and environmental settings. Traditional microbiological techniques remain useful for isolation and preliminary identification of bacteria; however, they provide only a limited view of the genetic characteristics of bacterial isolates. Phenotypic antimicrobial susceptibility testing, for example, can establish whether an organism is resistant to particular antimicrobial agents but cannot comprehensively determine the genetic basis of resistance. Whole-genome sequencing (WGS) provides a considerably broader approach because it permits simultaneous investigation of bacterial identity, antimicrobial resistance genes, virulence-associated genes, plasmid-associated determinants, sequence types and genetic relatedness. WGS can therefore provide a more detailed understanding of the epidemiology and transmission potential of bacterial organisms within livestock production systems. Recent studies have demonstrated the value of WGS in investigating bacteria from pig production systems. In a South African commercial pig farm, whole-genome characterization of *E. coli* isolates from pigs and human contacts identified substantial antimicrobial resistance and a diversity of resistance genes, while also revealing shared sequence types among isolates from different hosts [7]. Similarly, Vilaró *et al*. [8] used WGS to investigate *E. coli* within vertically integrated pig production systems and reported considerable genetic diversity alongside resistance to several important antimicrobial classes. These findings demonstrate how genomic surveillance can move beyond simply determining whether an organism is resistant and can provide information about the genetic composition, epidemiological relationships and potential movement of resistant organisms. The application of WGS is particularly valuable within a One Health framework because bacterial genomes can provide evidence linking organisms recovered from different ecological compartments. Comparative genomic analysis may reveal closely related isolates, shared resistance determinants or mobile genetic elements that suggest possible transmission pathways. Such information is important in pig production systems where bacteria may circulate among pigs, farm workers, manure, water, soil and other environmental reservoirs. The WHO [4] has emphasized that effective AMR surveillance must consider environmental pathways alongside human and animal reservoirs, while Woolhouse [2] highlighted the importance of understanding the relative contributions of these interconnected compartments to the emergence and persistence of resistance.

The One Health concept recognizes that human, animal and environmental health are interdependent and that complex health threats require collaboration across disciplinary and sectoral boundaries. This approach is particularly appropriate for AMR because resistant organisms and resistance genes do not remain confined to the setting in which they emerge. Instead, they can move between humans, animals and environmental reservoirs through multiple pathways [1, 2]. For Nigeria, where livestock production is expanding alongside continuing challenges in antimicrobial stewardship and environmental management, One Health surveillance can provide a framework for identifying emerging bacterial hazards before they become more widely

established. Against this background, the present study focuses on the whole-genome characterization of Gram-negative bacteria in a pig production system at Okada, Edo State, Nigeria, employing a One Health approach**.** The study is designed to provide genomic information on the bacterial organisms present within the pig production environment. By combining bacterial characterization with genomic analysis, the study is expected to generate information that can contribute to understanding the circulation of Gram-negative bacteria within pig production systems.

2. Materials and Methods

2.1 Study Area

The study was conducted in Okada, Ovia South-West Local Government Area of Edo State, Nigeria. Okada is a rural and semi-rural community in Edo State where agricultural activities, including livestock production, form an important component of local livelihoods. Pig production systems in the area comprise animal housing facilities, feeding areas, manure accumulation sites, drainage channels and other environmental compartments that can facilitate interaction between livestock, farm workers and the surrounding environment.

2.2 Study Design

A cross-sectional descriptive study was conducted to characterize Gram-negative bacteria associated with the pig production system at Okada, Edo State, Nigeria. Samples were collected from pigs, pig-associated farm environments and human-associated components of the production system. The sampling framework was designed to capture bacterial populations occurring at different points of contact within the pig production system. The principal sampling units included: pig skin and faecal samples; pig-associated environmental samples such as wastewater, soil, and feeds collected from piggery environments; as well as urine and nasal swab samples of consenting farm workers and other humans in close association with the piggery environment. The bacterial isolates were recovered using standard microbiological procedures and identified using conventional and molecular techniques. Representative Gram-negative isolates undergone whole-genome sequencing and genomic data were analyzed to determine bacterial identity, thereby providing a One Health assessment of bacterial characterization within the production system.

2.3 Ethical Considerations and Biosafety

Ethical approval was obtained from Igbinedion University, Okada (IUO) research ethics committee before commencement of the study. Informed consent was also obtained from participating farm owners or managers. Participants and farm workers were also informed about the purpose of the investigation, the nature of the specimens required, potential risks and benefits, confidentiality measures and their right to withdraw from the study without penalty. No personal identifiers were incorporated into laboratory sample codes or genomic datasets. Human-associated information was treated confidentially and used only for the purposes approved by the ethics committee.

2.4 Collection and Transportation of Samples

Sterile containers and sterile swabs were used according to sample type. The samples were collected aseptically to minimize contamination from external sources. Faecal samples were collected from rectal sampling procedures. Environmental samples were collected from relevant sites such as piggery-associated soils, feeds, and drainage or waste-disposal points. Samples were immediately labelled and placed in a cooled transport container. Samples were transported to the microbiology laboratory and processed within four hours of sample collection to preserve bacterial viability and reduce changes in the microbial population.

2.5 Culture Enrichment and Isolation of Gram-Negative Bacteria

Culture enrichment involved inoculating samples into buffered peptone water enrichment broths and incubating them overnight at 35 ºC suitable for the recovery of Gram-negative bacteria [9, 10]. Following enrichment, aliquots were streaked onto Chromocult Tryptone Bile-glucuronide agar medium (Techno Pharmchem Ltd, India) for the primary isolation and differentiation of Gram-negative bacteria. After inoculation, the culture plates were incubated at 35 ºC for 18 to 24 hours. Following incubation, colonies displaying different morphological characteristics were selected for purification. Colony morphology, pigmentation, lactose fermentation, mucoid appearance, and other observable characteristics were recorded. Distinct colonies were repeatedly subcultured until pure cultures were obtained. Pure isolates were maintained andstored in Nutrient Agar slants (HiMedia, India) at 4 ºCfor subsequent phenotypic and molecular analyses.

2.6 Identification of Bacterial Isolates

Preliminary identification was performed using standard microbiological procedures [11, 12]. Isolates were examined by Gram staining and evaluated using appropriate biochemical tests. The biochemical identification scheme included relevant tests such as: lactose fermentation, indole, citrate utilization, methyl red, and Voges–Proskauer. Results were interpreted using standard bacteriological identification criteria. Because phenotypic identification can occasionally fail to distinguish closely related members of the Enterobacterales and other Gram-negative groups, genomic identification was subsequently used to provide higher-resolution taxonomic assignment.

2.7 Genomic DNA Extraction

Genomic DNA of the freshly cultured bacterial broth was extracted using a validated commercial bacterial genomic DNA extraction kit following the manufacturer's instructions (Zymo Research Corporation, USA).

2.8 Assessment of DNA Quantity and Quality

Extracted genomic DNA was evaluated for both concentration and purity before sequencing.DNA concentration was determined using a fluorometric method, while purity was assessed spectrophotometrically using absorbance ratios. DNA integrity was also evaluated using

agarose gel electrophoresis. Only DNA preparations meeting the sequencing provider's or library-preparation manufacturer's quality requirements were advanced to sequencing.

2.9 Whole-Genome Shotgun Library Preparation

Genomic DNA was quantified fluorometrically with a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) using a target specific Qubit assay (dsDNA BR Assay Kit, Thermo Fisher Scientific).The Illumina Nextera XT DNA library preparation kit (Illumina Inc, San Diego, CA, USA) was used for preparation of fragment libraries of the bacterial genomes, and index codes were added to attribute sequences to each sample, as previously described [13] . A total of 1ng of genomic DNA per sample was used for DNA fragment library preparation. Briefly, the DNA sample was fragmented by sonication to a size of 350bp, then DNA fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing, with further PCR amplification. The PCR products were ultimately purified (AMPure XP system) and libraries were analyzed for size distribution by Agilent2100 Bioanalyzer and quantified using real-time PCR. The clustering of the index-coded samples was performed on a cBot Cluster Generation System according to the manufacturer's instructions.

2.10 Whole-Genome Shotgun Sequencing

The prepared libraries were subjected to whole-genome shotgun sequencing using a high-throughput sequencing platform. Sequencing generated paired-end short reads where an Illumina-type platform was used [13, 14]. Briefly, the library preparations were sequenced on an Illumina HiSeq platform and paired-end reads (2 × 350 bp) were generated. Raw sequence data were generated and FASTQ files were obtained. The quality of obtained data was assessed using FASTQC (v.0.11.5) and low-quality reads were filtered out and adapter regions were removed using Trimmomatic (v.0.36) [15]. The raw reads obtained from sequencing were assembled denovo using MEGAHIT version 1.2.9 [16], and the quality of assembled genome was determined through Quality Assessment Tool (QUAST) v5.0.2 [17]. The assembled contigs were deposited in the National Center for Biotechnological Information (NCBI) repository.

2.11 Sequencing Quality Control

Raw sequencing reads were subjected to quality assessment before downstream analysis. Quality-control procedures included assessment of: per-base sequence quality ,per-sequence quality scores, GC content, sequence length distribution, adapter contamination, duplicated reads, and other indicators of sequencing quality. FastQC was used for initial read-level quality. FastQC produces detailed quality reports for high-throughput sequencing datasets, allowing problematic libraries to be identified before downstream analysis. Low-quality bases and sequencing adapters were removed using an appropriate read-trimming program [Trimmomatic (v.0.36)]. Reads that remained below the predetermined quality or length threshold after trimming were discarded.

2.12 De novo Genome Assembly

High-quality sequencing reads were assembled de novo to reconstruct draft bacterial genomes. A short-read assembler (MEGAHIT version 1.2.9) appropriate for bacterial WGS data was used. Assembly quality was assessed using parameters including: total assembly length, number of contigs, N50, L50, largest contig, and GC content. Assemblies with substantial contamination, excessive fragmentation or otherwise inadequate quality were excluded from downstream comparative analyses or subjected to additional processing.

2.13 Taxonomic Classification of Sequencing Reads

Taxonomic classification was performed to evaluate the composition and identity of the bacterial sequence data. Since the culture-enriched samples contained mixed sequence reads, a k-mer-based taxonomic classifier, Kraken 2**,** was used to classify reads against a curated reference database. Bracken was then used to estimate taxonomic abundance from Kraken-classified reads. Because the principal objective was characterization of cultured bacterial isolates, taxonomic classification was subsequently reconciled with the original culture-based identification.

2.14 Statistical Analysis

Phenotypic data were entered into a spreadsheet and subsequently analyzed using Number Cruncher Statistical Software (NCSS) ver. 12 data analysis software. Descriptive statistics were used to summarize frequency of bacterial species while Pearson chi-square test of independence, goodness-of-fit analysis, Cochran's Q and Jaccard tests were used to evaluate the bacterial distribution. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Distribution of samples examined from the pig production system

A total of 356 samples were examined from the pig production system at Okada, Edo State, representing the three principal components of the One Health framework: animal, environmental and human-associated sources (Table 1). Pig-associated samples constituted the largest proportion of the samples collected, with 132 samples (37.08%) obtained from faecal, rectal and skin sources. Environmental samples accounted for 124 samples (34.83%), comprising wastewater, soil and feed, while 100 human-associated samples (28.09%) were obtained from farm workers and other individuals who had close contact with the piggery environment. The relatively close distribution of samples among the three compartments indicates that the investigation did not focus exclusively on pigs but incorporated the wider ecological setting in which pig production occurs. Animal samples represented the principal biological reservoir, whereas environmental samples captured potential pathways through which microorganisms and resistance determinants could persist or circulate within the production system. Human-associated samples provided an additional component for assessing the interface between occupational exposure and the piggery environment.

Table 1. Distribution of samples collected from the pig production system

One Health compartment	Sample type	Number of samples (n)	Percentage (%)
Pig-associated	Faecal, rectal and skin samples	132	37.08
Environmental	Wastewater, soil and feed samples	124	34.83
Human-associated	Farm workers and other persons associated with piggery environments	100	28.09
Total		356	100.00

3.2 Phenotypic Identification and frequency of occurrence of Gram-Negative Bacteria

Following cultural examination, Gram staining and biochemical characterization (Table 2), Fourty-eight (48) Gram-negative bacterial isolates were recovered from the pig production system. The isolates originated from the three One Health compartments: pig-associated samples, environmental samples and human-associated samples. Pig-associated samples yielded the highest number of isolates (22; 45.83%), followed by environmental samples (14; 29.17%) and human-associated samples (12; 25.00%).The isolates were phenotypically characterized based on colonial appearance on nutrient agar, Gram reaction, citrate utilization, indole production, methyl red reaction, Voges–Proskauer reaction and lactose fermentation. The isolates were predominantly Gram-negative rods, with most producing mucoid colonies. The major genera identified were *Proteus*, *Enterobacter*, *Pseudomonas*, *Escherichia*, *Klebsiella*, *Bordetella*, *Morganella*, *Providencia* and *Citrobacter*. All 48 isolates demonstrated the characteristic Gram reaction of Gram-negative bacteria and appeared as Gram-negative rods on microscopic examination. Most isolates produced mucoid colonies on nutrient agar. The principal exception was *Pseudomonas* spp., for which green pigmentation. The predominance of mucoid colonies was particularly evident among the Enterobacterales, including; *Klebsiella*, *Enterobacter*, *Escherichia*, *Proteus*, *Providencia* and *Morganella*. The colonial and microscopic characteristics provided an initial basis for grouping the isolates before biochemical differentiation. The observation of green colonies among *Pseudomonas* isolates also provided a useful presumptive characteristic for separating these organisms from the predominantly mucoid Enterobacterales. The biochemical reactions demonstrated considerable phenotypic differentiation among the isolates. *Proteus* spp. were citrate positive, indole negative and lactose negative, with variable methyl red reactions. *Providencia* spp. were citrate and indole positive and methyl-red positive but VP and lactose negative. *Morganella* spp. were citrate negative, indole positive and methyl-red positive, with negative VP and lactose reactions.The *Enterobacter* and *Klebsiella* isolates showed a broadly similar biochemical profile, characterized by citrate positivity, indole negativity, VP positivity and lactose fermentation. *Escherichia* spp. were predominantly indole positive and methyl-red positive but citrate and VP negative. *Pseudomonas* spp. showed citrate-positive reactions with negative indole, MR, VP and lactose reactions. *Citrobacter* spp. were citrate positive, indole negative, MR negative, VP negative and lactose positive. These reaction patterns were used collectively for presumptive phenotypic identification rather than relying on a single biochemical test.Across all three compartments, *Proteus* spp. were the most

frequently recovered organisms, accounting for 13 of 48 isolates (27.08%). *Enterobacter* spp. were the second most abundant (7; 14.58%), followed by *Pseudomonas* spp. (6; 12.50%). *Klebsiella*, *Escherichia* and *Bordetella* each accounted for 5 isolates (10.42%). *Morganella* and *Providencia* each contributed 3 isolates (6.25%), while *Citrobacter* spp. was the least abundant (1; 2.08%) (Table 3)

3.3 Distribution of Gram-negative bacteria

The distribution of Gram-negative bacteria in all three compartments is presented in Table 3. The distribution indicates that several bacterial genera occurred across more than one compartment. *Proteus*, *Enterobacter*, *Pseudomonas*, *Klebsiella*, *Escherichia*, *Morganella* and *Providencia* were recovered from all the three compartments. *Bordetella* spp. was recovered exclusively from pig-associated samples, whereas *Citrobacter* spp. occurred both in the environmental and human compartments. A Pearson chi-square test of independence was used to determine whether the distribution of bacterial genera was significantly associated with the One Health compartment. There was no statistically significant association between bacterial genus and compartment ($\chi^2 = 11.25$, df = 16, $p = 0.79$). Thus, although the numerical abundance of individual genera varied between pig-associated, environmental and human-associated samples, the observed distribution did not provide statistical evidence that bacterial genus was dependent on compartment. A separate goodness-of-fit analysis indicated that the overall abundance of the nine bacterial genera was not evenly distributed ($\chi^2 = 4.00$, df = 2, $p = 0.135$). This indicates significant variation in the overall abundance of the identified genera, with *Proteus* spp. occurring considerably more frequently than *Citrobacter*, *Morganella* and *Providencia* spp. The predominance of *Proteus* across the One-Health compartments suggests possible ecological connectivity, emphasizing the need for integrated surveillance and improved hygiene, waste management, and prudent antimicrobial use within pig production systems.

Table 2. Cultural, morphological, biochemical characteristics and frequency of occurrence of the Gram-negative bacteria

NA: Nutrient agar; CI: Citrate test. IN: Indole test: MR: Methyl red test. VP: Voges Proskauer test; LA: Lactose test: +

One Health compartment n (%)	Colonial and morphological characteristics		Biochemical tests					Probable bacteria	Frequency of occurrence of bacteria n (%)
	Growth on NA Petri plates	Gram-staining	CI	IN	MR	VP	LA		
Pig-associated 22 (45.83)	Mucoid colonies	Negative rods	+	-	V	-	-	*Proteus* spp.	5 (22.73)
	Mucoid colony	Negative rods	+	+	+	-	-	*Providencia* spp.	1 (4.55)
	Mucoid colony	Negative rods	-	+	+	-	-	*Morganella* spp.	1 (4.55)
	Mucoid colonies	Negative rods	+	-	-	+	+	*Enterobacter* spp.	3 (13.63)
	Mucoid colonies	Negative rods	-	+	+	-	+	*Escherichia* spp.	2 (9.09)
	Mucoid colonies	Negative rods	+	-	-	+	+	*Klebsiella* spp.	2 (9.09)
	Green colonies	Negative rods	+	-	-	-	-	*Pseudomonas* spp.	3 (13.63)
	Mucoid colonies	Negative rods	+	-	-	-	-	*Bordetella* spp.	5 (22.73)
Environmental 14 (29.17)	Mucoid colonies	Negative rods	+	-	V	-	-	*Proteus* spp.	4 (28.57)
	Mucoid colonies	Negative rods	+	-	-	+	+	*Klebsiella* spp.	2 (14.29)
	Mucoid colonies	Negative rods	-	+	+	-	+	*Escherichia* spp.	1 (7.14)
	Mucoid colonies	Negative rods	+	-	+	-	+	*Citrobacter* spp.	1 (7.14)
	Mucoid colonies	Negative rods	+	-	-	-	-	*Pseudomonas* spp.	2 (14.29)
	Mucoid colony	Negative rods	-	+	+	-	-	*Morganella* spp.	1 (7.14)
	Mucoid colony	Negative rods	+	+	+	-	-	*Providencia* spp.	1 (7.14)
	Mucoid colonies	Negative rods	+	-	-	+	+	*Enterobacter* spp.	2 (14.29)
Human-associated 12 (25)	Mucoid colonies	Negative rods	+	-	V	-	-	*Proteus* spp.	4 (33.33)
	Mucoid colonies	Negative rods	+	-	-	+	+	*Klebsiella* spp.	1 (8.33)
	Mucoid colonies	Negative rods	-	+	+	-	+	*Escherichia* spp.	2 (16.67)
	Mucoid colonies	Negative rods	+	-	-	-	-	*Pseudomonas* spp.	1 (8.33)
	Mucoid colonies	Negative rods	+	-	-	+	+	*Enterobacter* spp.	2 (16.67)
	Mucoid colony	Negative rods	-	+	+	-	-	*Morganella* spp.	1 (8.33)
Total = 48 (100)	Mucoid colony	Negative rods	+	+	+	-	-	*Providencia* spp.	1 (8.33)

: Positive results. -: Negative results; n: number; %: percentage.

Table 3. Distribution of Gram-negative bacterial genera

Bacterial genus	Total isolate n (%)	One Health compartments		
		Pig-associated	Environmental	Human-associated
Proteus	13 (27.08)	+	+	+
Providencia	3 (6.25)	+	+	+
Morganella	3 (6.25)	+	+	+
Enterobacter	7 (14.58)	+	+	+
Escherichia	5 (10.42)	+	+	+
Klebsiella	5 (10.42)	+	+	+
Bordetella	5 (10.42)	+	-	-
Citrobacter	1 (2.08)	-	+	+
Pseudomonas	6 (12.50)	+	+	+

+: presence of bacteria; -: absence of bacteria; n: number; %: percentage

3.4 Whole-Genome Sequencing and Quality Assessment of Gram-Negative Isolates

The sequencing and assembly metrics for the three One Health compartments are summarized in Tables 4 and 5. All Gram-negative isolates recovered from the different One Health compartments were subjected to culture-enriched whole-genome shotgun sequencing. The sequenced isolates consisted of 22 from pigs, 14 from the farm environment, and 12 from human-

associated samples. The sequencing libraries yielded mean numbers of 17,626,498, 20,085,052, and 23,028,031 high-quality reads for environmental, human-and pig-associated isolates, respectively, with mean Phred quality scores of at least Q30. Following quality-control filtering, 95.50% of the reads from environmental isolates, 96.50% from pig-associated isolates, and 96.00% from human-associated isolates were retained for subsequent bioinformatic analyses. The resulting genome assemblies showed mean N50 values of 21,521 bp for environmental isolates, 7,390 bp for pig-associated isolates, and 4,562 bp for human-associated isolates. The corresponding mean GC contents were 46.30%, 46.04%, and 42.58%, respectively. Overall, the whole-genome sequencing data generated a higher level of genomic resolution and discriminatory power than could be achieved through phenotypic characterization alone, thereby providing more detailed information for differentiating and characterizing the Gram-negative isolates across the One Health compartments.

Table 4. Whole Genome Sequence quality metrics of the culture-enriched metagenomic samples using FastQC report

Sample	Sequences flagged as poor quality after filtering	Clean sequence reads	Total bases (Gbp)	Sequence length	Q30 (%)
Pig-associated	0	23,028,031	3.40	50 – 150	96.50
Environmental	0	17,626,498	2.60	50 – 150	95.50
Human-associated	0	20,085,052	3.00	50 – 150	96.00

Gbp: gigabase pair

Table 5. Assembly statistics of the culture-enriched metagenomic samples using the MEGAHIT assembler

Sample	Total number of contigs	Total assembly length bp	Largest contig bp	Mean contig length bp	N50 bp	L50	GC content (%)
Pig-associated	19,873	57,864,453	527,694	2,911.71	7,390	1,138	46.04
Environmental	18,421	82,565,074	581,121	4,482.12	21,521	699	46.30
Human-associated	30,394	72,289,129	638,580	2,378.40	4,562	2,706	42.58

bp: nucleotide base pair

3.5 Genomic identification of sequenced isolates recovered from the pigs

The hierarchical taxonomic analysis of sequenced data obtained from pigs (Figure 1) revealed a diverse bacterial community dominated by Proteobacteria, particularly the class Gammaproteobacteria. Within this group, Enterobacterales was prominent, with *Escherichia*, *Klebsiella*, and *Enterobacter* representing important genera. *Escherichia coli* and *Klebsiella pneumoniae* each accounted for approximately 8% of classified sequences, while *Enterobacter hormaechei*, *E. cloacae*, and unclassified *Enterobacter* contributed smaller proportions. *Pseudomonas aeruginosa* represented approximately 6%, alongside other *Pseudomonas* sequences. Members of Morganellaceae, including *Morganella*, *Providencia*, and *Proteus*, were also detected, with several *Proteus* species showing relatively comparable abundances. A distinct *Bordetella* branch was identified, with *Bordetella trematum* being the most abundant explicitly labelled taxon at approximately 16%. Overall, the bacterial community demonstrated considerable taxonomic heterogeneity, with explicitly labelled taxa ranging from 2% to 16%. The relatively high abundance of *B. trematum* contributed to a positively skewed distribution, while numerous taxa occurred at lower proportions of 2–4%. The findings demonstrate that the bacterial population associated with the pigs was not restricted to a single taxonomic group but comprised diverse Gram-negative taxa with varying relative abundances.



Figure 1. **Krona chart showing the taxonomic classification and relative abundance Gram-negative bacteria isolated from pigs**

3.6 Genomic identification of sequenced isolates recovered from the Piggery Environment

The taxonomic composition of the bacteria recovered from the piggery environment was assessed using whole-genome shotgun sequencing data and visualized with a Krona hierarchical classification plot (Figure 2). Whole-genome shotgun sequencing of bacteria recovered from the piggery environment revealed a diverse and heterogeneous bacterial community, with sequences predominantly assigned to the phylum Proteobacteria, class Gammaproteobacteria, and order Enterobacterales. The major families identified were Enterobacteriaceae, Morganellaceae, Pseudomonadaceae, and Halomonadaceae. Within Enterobacteriaceae, *Escherichia coli*, *Citrobacter freundii*, and *Klebsiella variicola* were the most prominent taxa, each accounting for approximately 9% of the classified sequences. Other important taxa included *Klebsiella pneumoniae* (5%), *Enterobacter hormaechei* (3%), and *Enterobacter cloacae* (2%).

Morganellaceae was represented by *Proteus*, *Providencia*, and *Morganella*, with unclassified *Proteus* (6%), *Proteus rettgeri* (5%), *P. penneri* (5%), *P. mirabilis* (4%), and *Morganella morganii* (6%) contributing substantially to the profile. The Pseudomonadaceae family included *Pseudomonas qingdaonensis* (3%), unclassified *Pseudomonas* (3%), and *Pseudomonas putida* (2%). A small proportion of sequences was assigned to Halomonadaceae, represented by *Halomonas* (1%). Overall, the explicitly identified taxa displayed relative abundances ranging from 1% to 9%, with the three leading taxa collectively accounting for approximately 27% of the profile. The absence of a single overwhelmingly dominant species, together with the presence of numerous low-abundance and unclassified taxa, indicates substantial taxonomic diversity within the piggery environment.

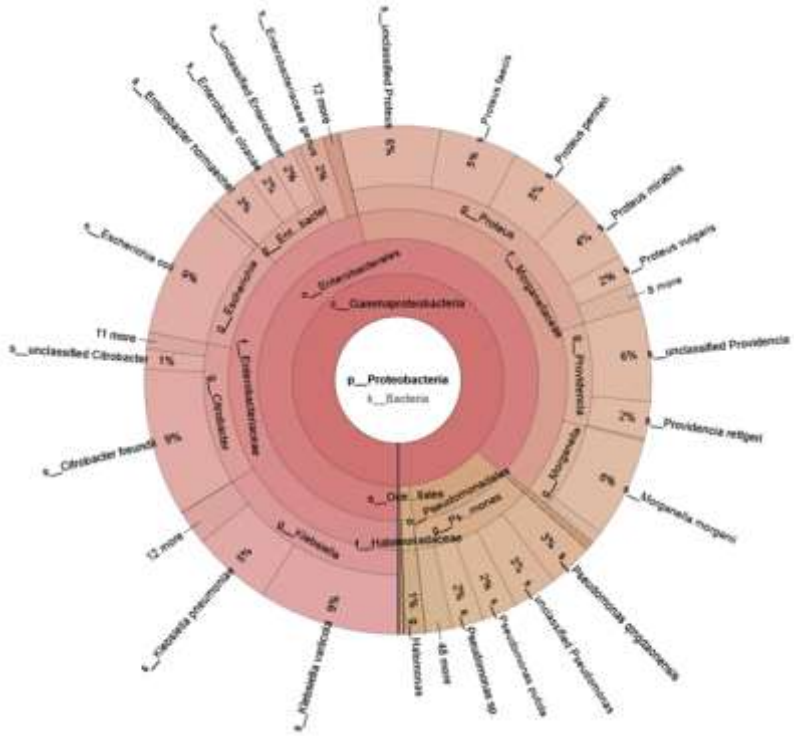


Figure 2. Krona chart showing the taxonomic classification and relative abundance of Gram-negative bacteria isolated from the piggery environment

3.7 Genomic identification of sequenced isolates recovered from the farm workers and humans living within the piggery environment

The hierarchical taxonomic classification of the sequenced bacterial population is presented as a Krona plot (Figure 3). Culture-enriched whole-genome shotgun sequencing of bacterial isolates recovered from farm workers and humans associated with the piggery environment revealed a diverse bacterial population dominated by Proteobacteria, particularly Gammaproteobacteria and the order Enterobacterales. The major families were Enterobacteriaceae and Morganellaceae, with *Pseudomonas* also represented. *Escherichia coli* was the most abundant explicitly identified taxon, accounting for approximately 14% of classified sequences, followed by *Enterobacter cloacae* (9%) and *Citrobacter freundii* (8%). Other notable taxa included *Morganella morganii* and unclassified *Providencia*, each contributing approximately 8%, while unclassified *Proteus* accounted for 7%. Several *Proteus* species were identified, including *P.*

penneri and *P. faecis* (6% each), as well as *P. vulgaris* and *P. mirabilis* (2% each). *Klebsiella pneumoniae* and *Pseudomonas* taxa occurred at lower proportions. Overall, the explicitly labelled taxa showed relative abundances ranging from 1% to 14%, indicating considerable variation in their representation. The bacterial community was characterized by a predominance of Enterobacterales-associated organisms, particularly members of Enterobacteriaceae and Morganellaceae. However, the presence of numerous additional low-abundance and unclassified taxa demonstrated substantial taxonomic diversity. The distribution indicated that the human-associated bacterial population was not dominated exclusively by a single genus but consisted of multiple bacterial taxa occurring at different relative abundances, highlighting the diverse microbial composition associated with humans within the piggery environment.



Figure 3. Krona chart showing the taxonomic classification and relative abundance of Gram-negative bacteria isolated from farm workers and humans living within piggery environment

3.8 NCBI accession characteristics of Gram-negative bacterial species isolated from the pigs

Whole-genome sequencing was used to identify the Gram-negative bacterial isolates recovered from pigs. As presented in Table 6, a total of 12 Gram-negative bacterial isolates were identified to species level. The isolates comprised members of the families Morganellaceae, Enterobacteriaceae, Pseudomonadaceae and Alcaligenaceae, demonstrating considerable taxonomic diversity among the Gram-negative bacteria recovered from the pig samples.The Morganellaceae constituted the largest group, comprising six isolates: *Proteus faecis* (AOC-BE22), *Proteus penneri* (AOC-BE23), *Proteus mirabilis* (AOC-BE24), *Proteus vulgaris* (AOC-BE25), *Providencia rettgeri* (AOC-BE26), and *Morganella morganii* (AOC-BE27). The

Enterobacteriaceae were represented by four species, namely *Enterobacter hormaechei* (AOC-BE28), *Enterobacter cloacae* (AOC-BE29), *Escherichia coli* (AOC-BE30), and *Klebsiella pneumoniae* (AOC-BE31). In addition, *Pseudomonas aeruginosa* (AOC-BE32) and *Bordetella trematum* (AOC-BE33) were identified, representing Pseudomonadaceae and Alcaligenaceae, respectively.Among the identified organisms, *Proteus* spp. were the most represented genus, with four species (*P. faecis, P. penneri, P. mirabilis* and *P. vulgaris*), accounting for one-third of the bacterial species identified. *Enterobacter* was represented by two species, while each of *Providencia, Morganella, Escherichia, Klebsiella, Pseudomonas* and *Bordetella* was represented by one species. The whole-genome sequencing data were deposited in the National Center for Biotechnology Information (NCBI) database. Individual isolates were associated with WGS accession-number ranges corresponding to their assembled contigs. The accession series ranged from JCAWQV010000001–JCAWQV010000013 for *P. faecis* to JCAWQW010000001–JCAWQW010000018 for *P. penneri*. The number of accessioned contigs varied among isolates, with *P. penneri* having the highest number of contigs (18), followed by *P. faecis*, *E. hormaechei* and *P. aeruginosa*, each with 13 contigs. *P. mirabilis* had the lowest number of contigs (6).The variation in the number of contigs among the isolates reflects differences in genome assembly characteristics rather than differences in the number of bacterial species present.

Table 6. Whole-genome identification and NCBI accession numbers of Gram-negative bacterial species isolated from pigs

Bacterial species	Isolate	NCBI WGS accession range
Proteus faecis	AOC-BE 22	JCAWQV010000001 to JCAWQV010000013
Proteus penneri	AOC-BE 23	JCAWQW010000001 to JCAWQW010000018
Proteus mirabilis	AOC-BE 24	JCAWQX010000001 to JCAWQX010000006
Proteus vulgaris	AOC-BE 25	JCAWQY010000001 to JCAWQY010000007
Providencia rettgeri	AOC-BE 26	JCAWQZ010000001 to JCAWQZ010000012
Morganella morganii	AOC-BE 27	JCAWRA010000001 to JCAWRA010000011
Enterobacter hormaechei	AOC-BE 28	JCAWRB010000001 to JCAWRB010000013
Enterobacter cloacae	AOC-BE 29	JCAWRC010000001 to JCAWRC010000010
Escherichia coli	AOC-BE 30	JCAWRD010000001 to JCAWRD010000008
Klebsiella pneumoniae	AOC-BE 31	JCAWRE010000001 to JCAWRE010000010
Pseudomonas aeruginosa	AOC-BE 32	JCAWRF010000001 to JCAWRF010000013
Bordetella trematum	AOC-BE 33	JCAWRG010000001 to JCAWRG010000009

NCBI: National Center for Biotechnology Information, United States

3.9 NCBI accession characteristics of Gram-negative bacterial species isolated from the piggery environment

The identified bacterial species, as shown in Table 7, comprised *Klebsiella variicola* (AOC-BE1), *Klebsiella pneumoniae* (AOC-BE2), *Citrobacter freundii* (AOC-BE3), *Escherichia coli* (AOC-BE4), *Enterobacter hormaechei* (AOC-BE5), *Enterobacter cloacae* (AOC-BE6),

Proteus faecis (AOC-BE7), *Proteus penneri* (AOC-BE8), *Proteus mirabilis* (AOC-BE9), *Proteus vulgaris* (AOC-BE10), *Providencia rettgeri* (AOC-BE11), *Morganella morganii* (AOC-BE12), *Pseudomonas qingdaonensis* (AOC-BE13), and *Pseudomonas putida* (AOC-BE14).The Enterobacteriaceae group comprised six species, namely *K. variicola, K. pneumoniae, C. freundii, E. coli, E. hormaechei,* and *E. cloacae*. The Morganellaceae group comprised six species, including *P. faecis, P. penneri, P. mirabilis, P. vulgaris, P. rettgeri,* and *M. morganii*. The remaining two isolates were *P. qingdaonensis* and *P. putida*, both belonging to the genus *Pseudomonas*. Thus, the isolates demonstrated representation from three major bacterial lineages within the Gram-negative bacterial community recovered from the piggery environment.

Table 7. Whole-genome identification and NCBI accession numbers of Gram-negative bacterial species isolated from piggery environment

Bacterial species	Isolate	NCBI Genome assembly accession numbers
Klebsiella variicola	AOC-BE1	JCAWQA010000001 to JCAWQA010000011
Klebsiella pneumoniae	AOC-BE2	JCAWQB010000001 to JCAWQB010000010
Citrobacter freundii	AOC-BE 3	JCAWQC010000001 to JCAWQC010000014
Escherichia coli	AOC-BE 4	JCAWQD010000001 to JCAWQD010000011
Enterobacter hormaechei	AOC-BE 5	JCAWQE010000001 to JCAWQE010000010
Enterobacter cloacae	AOC-BE 6	JCAWQF010000001 to JCAWQF010000009
Proteus faecis	AOC-BE 7	JCAWQG010000001 to JCAWQG010000014
Proteus penneri	AOC-BE 8	JCAWQH010000001 to JCAWQH010000014
Proteus mirabilis	AOC-BE 9	JCAWQI010000001 to JCAWQI010000011
Proteus vulgaris	AOC-BE 10	JCAWQJ010000001 to JCAWQJ010000011
Providencia rettgeri	AOC-BE 11	JCAWQK010000001 to JCAWQK010000004
Morganella morganii	AOC-BE 12	JCAWQL010000001 to JCAWQL010000016
Pseudomonas qingdaonensis	AOC-BE 13	JCAWQM010000001 to JCAWQM010000014
Pseudomonas putida	AOC-BE 14	JCAWQN010000001 to JCAWQN010000008

NCBI: National Center for Biotechnology Information, United States

3.10 NCBI accession characteristics of the human-associated bacterial isolates

The bacterial species in human-associated bacterial isolates identified through whole-genome sequencing, as shown in Table 8, comprised *Enterobacter cloacae* (AOC-BE43), *Enterobacter roggenkampii* (AOC-BE44), *Escherichia coli* (AOC-BE45), *Citrobacter freundii* (AOC-BE46), *Klebsiella pneumoniae* (AOC-BE47), *Proteus faecis* (AOC-BE48), *Proteus penneri* (AOC-BE49), *Proteus vulgaris* (AOC-BE50), *Proteus mirabilis* (AOC-BE51), *Providencia rettgeri* (AOC-BE52), *Morganella morganii* (AOC-BE53), and *Pseudomonas qingdaonensis* (AOC-BE54). The isolates represented 12 bacterial species distributed across several genera of Gram-negative bacteria recovered from humans within the piggery production system. The family Enterobacteriaceae comprised five species, namely *E. cloacae, E. roggenkampii, E. coli,*

C. freundii, and *K. pneumoniae*. The Morganellaceaegroup comprised six species, including *P. faecis, P. penneri, P. vulgaris, P. mirabilis, P. rettgeri,* and *M. morganii*. The remaining isolate, *P. qingdaonensis* (AOC-BE54), belonged to the genus *Pseudomonas*. Thus, the sequenced isolates demonstrated considerable taxonomic diversity, with representatives of three major Gram-negative bacterial lineages recovered from the pig production system. The whole-genome assemblies were deposited in the National Center for Biotechnology Information (NCBI) database under the corresponding contig accession ranges.

Table 8. Whole-genome identification and NCBI accession numbers of Gram-negative bacterial species isolated from farm workers and humans living within the vivinity of piggery environment

Bacterial species	Isolate	NCBI Genome assembly accession numbers
Enterobacter cloacae	AOC-BE 43	JCAWRQ010000001 to JCAWRQ010000018
Enterobacter roggenkampii	AOC-BE 44	JCAWRR010000001 to JCAWRR010000011
Escherichia coli	AOC-BE 45	JCAWRS010000001 to JCAWRS010000029
Citrobacter freundii	AOC-BE 46	JCAWRT010000001 to JCAWRT010000006
Klebsiella pneumoniae	AOC-BE 47	JCAWRU010000001 to JCAWRU010000005
Proteus faecis	AOC-BE 48	JCAWRV010000001 to JCAWRV010000022
Proteus penneri	AOC-BE 49	JCAWRW010000001 to JCAWRW010000013
Proteus vulgaris	AOC-BE 50	JCAWRX010000001 to JCAWRX010000003
Proteus mirabilis	AOC-BE 51	JCAWRY010000001 to JCAWRY010000008
Providencia rettgeri	AOC-BE 52	JCAWRZ010000001 to JCAWRZ010000005
Morganella morganii	AOC-BE 53	JCAWSA010000001 to JCAWSA010000027
Pseudomonas qingdaonensis	AOC-BE 54	JCAWSB010000001 to JCAWSB010000023

NCBI: National Center for Biotechnology Information, United States

3.11 Distribution of bacterial species across the One Health compartments

The distribution of bacterial species across the three compartments is presented in Table 9, and demonstrated considerable taxonomic overlap. A total of 17 bacterial species were identified, with the environmental compartment showing the highest species richness (14 species; 82.4%), compared with 12 species (70.6%) each in the pig-associated and human-associated compartments. Nine species (52.9%) were common to all three compartments, namely;*Proteus faecis*, *P. penneri*, *P. mirabilis*, *P. vulgaris*, *Providencia rettgeri*, *Morganella morganii*, *Enterobacter cloacae*, *Escherichia coli*, and *Klebsiella pneumoniae*. This widespread occurrence indicates substantial sharing of bacterial taxa among pigs, the environment, and humans. Several species showed compartment-specific distributions. *Pseudomonas aeruginosa* and *Bordetella trematum* were restricted to the pig-associated compartment, whereas *Enterobacter roggenkampii* occurred exclusively in the human-associated compartment. *Klebsiella variicola* and *Pseudomonas putida* were restricted to the environmental compartment, while *Citrobacter freundii* and *Pseudomonas qingdaonensis* occurred in both environmental and human-associated

samples. Statistical analysis using Cochran's Q test showed no significant difference in the proportion of bacterial species represented across the three compartments ($Q = 1.14$, $df = 2$, $p = 0.57$). Although the environmental compartment recorded the greatest numerical species richness, this difference was not statistically significant at the 5% level. Pairwise Jaccard similarity analysis further demonstrated substantial taxonomic overlap. The pig-associated and environmental compartments shared 10 species, producing a Jaccard coefficient of 0.66, while pig-associated and human-associated compartments shared nine species ($J = 0.60$). The highest similarity occurred between environmental and human-associated compartments, which shared 11 species ($J = 0.73$). Overall, the findings indicate broad distribution and substantial taxonomic connectivity of bacteria across the pig production system, with the environment potentially serving as an important interface linking animal and human-associated bacterial populations.

Table 9. Distribution of the Gram-negative bacterial species

Bacterial species	One Health compartments		
	Pig-associated	Environmental	Human-associated
Proteus faecis	+	+	+
Proteus penneri	+	+	+
Proteus mirabilis	+	+	+
Proteus vulgaris	+	+	+
Providencia rettgeri	+	+	+
Morganella morganii	+	+	+
Enterobacter hormaechei	+	+	
Enterobacter cloacae	+	+	+
Escherichia coli	+	+	+
Klebsiella pneumoniae	+	+	+
Pseudomonas aeruginosa	+	-	-
Bordetella trematum	+	-	-
Klebsiella variicola	-	+	-
Citrobacter freundii	-	+	+
Pseudomonas qingdaonensis	-	+	+
Pseudomonas putida	-	+	-
Enterobacter roggenkampii	-	-	+

Discussion

The present study investigated the distribution and diversity of Gram-negative bacteria within a pig production system using a One Health framework that incorporated pig-associated, environmental and human-associated compartments. A total of 356 samples were examined, comprising 37.08% animal-associated samples, 34.83% environmental samples and 28.09% human-associated samples. This sampling design provided an integrated basis for assessing bacterial occurrence across interconnected components of the pig production system. The inclusion of pigs, their surrounding environment and humans was particularly relevant because

antimicrobial resistance (AMR) is increasingly recognized as a problem that extends beyond individual sectors. Ohia *et al*. [18] emphasized that multidrug-resistant bacterial infections in Nigeria are influenced by interactions among human, animal and environmental systems, while Adebisi *et al*. [19] highlighted infection prevention, antimicrobial stewardship and environmental factors as important components of AMR control. Similarly, Al-Khalaifah *et al*. [20] described AMR as a problem involving interconnected reservoirs in humans, animals and the environment, with resistance genes capable of spreading through horizontal gene transfer. The study used culture-enriched whole-genome shotgun sequencing and Krona-based taxonomic classification to characterize the bacterial communities recovered from the piggery environment, pigs and farm workers or humans living within the piggery environment. Across the three compartments, the bacterial profiles were dominated by members of the phylum Proteobacteria, particularly Gammaproteobacteria and the order Enterobacterales. Important genera repeatedly identified included *Escherichia, Klebsiella, Enterobacter, Citrobacter, Proteus, Providencia* and *Morganella*. The repeated occurrence of these organisms is biologically significant because many Enterobacterales are capable of occupying intestinal, environmental and extraintestinal niches and may serve as reservoirs of antimicrobial-resistance genes. Musicha *et al*. [21] and Djordjevic *et al*. [22] emphasized that bacterial dissemination and AMR cannot be adequately understood by examining human, animal or environmental populations independently. Whole-genome sequencing therefore provides an important advantage by allowing bacterial organisms and resistance determinants to be investigated at a higher level of resolution than conventional phenotypic identification. The pig-associated Krona profile was dominated by Proteobacteria and Gammaproteobacteria, with Enterobacterales representing a substantial component of the classified bacterial community. *Bordetella trematum* was the most prominent explicitly identified organism, accounting for approximately 16% of the classified abundance, followed by *Escherichia coli* and *Klebsiella pneumoniae*, each representing approximately 8%. Other notable organisms included *Morganella morganii*, unclassified *Providencia*, *Pseudomonas aeruginosa* and unclassified *Proteus*, each at approximately 6%. Several *Proteus* species, including *P. mirabilis, P. penneri, P. vulgaris* and *P. hauseri*, were also identified. The occurrence of *E. coli, K. pneumoniae, Enterobacter, Morganella, Providencia* and *Proteus* among pig-associated bacteria is important because these organisms can potentially move from animals into manure, wastewater, dust and other environmental matrices. Linh *et al*. [23], in their investigation of paired faecal samples from pigs and livestock caretakers on Vietnamese smallholder farms, reported differences between pig and human microbiota despite considerable overlap in resistance-associated features. Similarly, Bai *et al*. [24] provided evidence suggesting that pig faeces may contribute to AMR gene profiles detected among piggery workers. The environmental compartment contained a particularly heterogeneous bacterial community involving *Escherichia, Citrobacter, Klebsiella, Enterobacter, Proteus, Providencia, Morganella* and *Pseudomonas*. Environmental samples are important in a One Health investigation because wastewater, soil, manure, feed, contaminated surfaces and other farm materials can serve as reservoirs or pathways for bacterial persistence and dissemination. Martak *et al*. [25] emphasized that animal manure and human-derived effluents can contribute to environmental contamination with resistant bacteria and resistance genes, particularly where

sanitation infrastructure is inadequate. Gudda *et al*. [26] similarly demonstrated the importance of environmental monitoring in resource-constrained livestock systems, while Lumabao *et al*. [27] reported the occurrence of antimicrobial-resistance genes across smallholder swine farms. These observations reinforce the importance of considering the farm environment as an active component of bacterial and AMR surveillance rather than simply as a passive recipient of animal contamination. The human-associated Krona profile was also dominated by Gammaproteobacteria and Enterobacterales. Particularly prominent organisms included *E. coli*, *E. cloacae*, *C. freundii*, *M. morganii*, unclassified *Providencia* and *Proteus*. The presence of these organisms among farm workers and other human-associated samples is epidemiologically relevant because individuals working or living within pig production environments may experience repeated contact with pigs, manure, contaminated surfaces, wastewater, soil, feed, equipment and environmental dust. Hibbard *et al*. [28] emphasized that agricultural systems can influence human health through direct animal contact, contaminated environments, food chains and antimicrobial-resistance pathways. Chen *et al*. [29] also showed that exposure to livestock-associated environments can influence the human microbiome and resistome, while Linh *et al*. [23] demonstrated considerable overlap in resistance determinants between pigs and livestock workers. Bai *et al*. [24] further reported evidence linking pig faecal AMR genes with those detected among workers. An important finding was the extensive overlap of bacterial species among the three compartments. Seventeen species were identified overall, with 12 species occurring in the pig-associated compartment (70.6%), 14 in the environmental compartment (82.4%) and 12 in the human-associated compartment (70.6%). Although the environmental compartment was numerically the richest, the difference in species representation was not statistically significant. Cochran's Q test produced $Q = 1.00$, $df = 2$ and $p = 0.607$, indicating that the differences among the three compartments were not statistically significant at the 5% significance level. Therefore, the environmental compartment can appropriately be described as having the greatest numerical species representation, but it cannot be concluded that it was significantly more species-rich than the pig-associated or human-associated compartments. The most important ecological observation was therefore not the difference in species richness but the extent of species sharing. Nine species, representing 52.9% of the total species identified, occurred in all three compartments. These included *Proteus faecis, P. penneri, P. mirabilis, P. vulgaris, P. rettgeri, Morganella morganii, Enterobacter cloacae, Escherichia coli* and *Klebsiella pneumoniae*. The environmental and human-associated compartments showed the highest pairwise similarity, sharing 11 species and producing a Jaccard coefficient of 0.733. The pig-associated and environmental compartments shared 10 species, producing a Jaccard coefficient of 0.625, whereas the pig-associated and human-associated compartments had a coefficient of 0.600. Pandey *et al*. [30] described livestock, human and environmental systems as interconnected reservoirs that can support the emergence and dissemination of AMR, and Pandey *et al*. [30] also emphasized that AMR transmission involves feedback mechanisms among human, animal and environmental systems. The high similarity between the pig-associated and environmental compartments is consistent with the close biological relationship between pigs and their immediate surroundings. Pigs continuously deposit faeces, urine, respiratory secretions and other biological materials into their environment, while manure and

wastewater can contaminate floors, drains, soil, water and equipment. At the same time, environmental microorganisms may enter pigs through contaminated feed, water, soil, surfaces and other farm materials. Consequently, the Jaccard coefficient of 0.625 should be interpreted as evidence of microbial connectivity rather than definitive proof of animal-to-environment transmission. Pandey *et al*. [30] emphasized that AMR is influenced by interconnected mechanisms involving livestock production, human activities and environmental contamination, while Martak *et al*. [25] identified manure, wastewater and other effluents as important potential vehicles for resistant organisms and resistance genes. The environmental-human similarity was even greater, with 11 shared species and a Jaccard coefficient of 0.73. Organisms such as *E. coli, K. pneumoniae, P. mirabilis, C. freundii* and *P. qingdaonensis* occurred in both compartments. This pattern may reflect continuous interaction between humans and contaminated environmental matrices, particularly in settings where sanitation and waste-management infrastructure are limited. Martak *et al*. [25] noted that inadequate sanitation and intensive animal farming can increase opportunities for movement of resistant organisms and resistance genes among animal, environmental and human reservoirs. Nevertheless, the high environmental-human similarity should not be interpreted as evidence that the environment was the source of human-associated bacteria. Because the study was cross-sectional, directionality cannot be established. Human-associated bacteria may have contributed to environmental contamination, just as environmental exposure may have contributed to human colonization. Five species showed restricted distributions. *Pseudomonas aeruginosa* and *Bordetella trematum* were detected only in the pig-associated compartment, *Klebsiella variicola* and *Pseudomonas putida* occurred only in the environmental compartment, while *Enterobacter roggenkampii* was detected only among human-associated samples. However, these restricted distributions should be interpreted cautiously because absence from a particular compartment does not establish ecological specificity. Detection can be influenced by sample size, sampling strategy, culture conditions and identification sensitivity. The detection of *K. variicola* in the environment is noteworthy because whole-genome approaches can help distinguish members of the closely related *Klebsiella* complex. Similarly, the detection of *B. trematum* exclusively in pigs may represent limited recovery rather than genuine ecological restriction. Consequently, the study provides an important epidemiological foundation for understanding the pig production system as a One Health microbial network and for designing future investigations into the emergence, persistence and transmission of antimicrobial-resistant Gram-negative bacteria.

Conclusion

The study demonstrated that the pig production system at Okada, Edo State, Nigeria, harboured a diverse assemblage of Gram-negative bacteria across pig-associated, environmental and human-associated compartments. Forty-eight isolates representing nine genera were recovered, with *Proteus* spp. predominating. Whole-genome shotgun sequencing provided greater taxonomic resolution than conventional phenotypic methods and revealed substantial representation of Proteobacteria, Gammaproteobacteria and Enterobacterales. Although bacterial distribution did not differ significantly among compartments ($\chi^2 = 11.25$, df = 16, $p = 0.79$), considerable species overlap was observed, with nine species occurring across all three

compartments. The findings highlight microbial connectivity within the pig production system and support integrated One Health surveillance, incorporating genomic and environmental monitoring approaches.

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