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Molecular identification of bacteria in ready-to-eat cow intestines from Ado-Ekiti Abattoir, Nigeria, Using 16S rRNA Sequencing

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Abstract

Background: Abattoir meat, particularly ready-to-eat cow intestine, is a valuable source of nutrients but may also harbor pathogenic microbes that compromise food safety. The present study investigated bacterial contaminants of freshly slaughtered cow intestine obtained from an abattoir in Ado-Ekiti, Nigeria, using 16S rRNA sequencing. Methods: Samples of freshly slaughtered cow intestines were aseptically selected and subjected to bacteria isolation. DNA was extracted, amplified by PCR for 16S rRNA gene, and sequenced. Results: Various bacterial species were found, including *Providencia vermicola* (97.74% and 95.98% similarity), *Pseudomonas pseudoalcaligenes* (99.93%), *Comamonas testosterone* (84.71%), *Escherichia coli* (99.32%), *Pseudomonas mendocina* (99.77%), *Bacillus subtilis* (99.78%), and *Comamonas kerstersii* (99.77%). The presence of opportunistic and pathogenic bacteria is of public health significance for meat processing hygiene. Conclusion: Freshly slaughtered cow intestines in Ado-Ekiti abattoir are harboring diverse populations of bacteria, including potential pathogens. Improved abattoir hygiene, strict regulatory enforcement, and consumer education are paramount to reducing foodborne risks.

Keywords: 16S RNA Sequencing; Abattoir Meat; Bacterial Contaminants; Public Health

1. Introduction

Foodborne diseases continue to loom large as a grave threat to public health worldwide (Gutema et al., 2021; Bedane et al., 2024; Uzoigwe et al., 2021; Olaitan et al., 2025). WHO figures suggest that nutrition-related pathogens cause hundreds of millions of illnesses each year, resulting in high rates of hospitalization, avoidable deaths, and substantial economic damages. For developing countries, and especially the countries of sub-Saharan Africa, the challenge is magnified by inadequate infrastructure, weak oversight, and a pressing need for affordable protein (Uzoigwe et al., 2021; Odetokun et al., 2023). Across the continent, beef remains a crucial protein source, yet the route from the slaughter house to the kitchen is fraught with unsanitary pathways where harmful microbes thrive (Bhutia et al., 2021; Makinde et al., 2021).

Within that protein supply, a particularly common item is cow intestine, enjoyed as “shaki” in Nigerian households and widely incorporated into soups and stews. The offal carries distinct culinary and nutritional value, yet the microbiological risks unopened by improper handling in meat markets should be taken seriously. Municipal slaughter facilities in cities like Ado-Ekiti and their suburbs routinely process cattle on dirt floors, rinse off entrails with untreated surface water, and rely on knives and containers that are seldom disinfected. Consequently, this environment due to activities stated above provides ideal conditions for the transmission of pathogenic and opportunistic microorganisms.

Meat microbial contamination research has largely highlighted *Escherichia coli, Salmonella, Listeria monocytogenes*, and *Staphylococcus aureus*, threats that remain perennial global problems, yet the microbial population in the neglected abattoir setting of southwest Nigeria has received scant investigation (Gutema et al., 2021; Olaitan et al., 2025; Odetokun

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et al., 2023). Traditional culture-based protocols, while valuable, bias isolation toward dominant or resilient species and overlook fastidious and yet-to-be-cultured microbes. Recent applications of molecular biology, particularly amplicon-based sequencing of the 16S rRNA gene, overcome this limitation by delivering simultaneous insight into whole microbial assemblages with minimal lag time and high throughput.

The 16S rRNA gene is both conserved and amenable to phylogenetic resolution because of discrete hypervariable regions. PCR amplification of this locus and subsequent sequencing therefore provides a robust genomic fingerprint across phylogenetic divides. Search and comparison to reference databases, for instance the extensively annotated NCBI GenBank, facilitate near real-time assignment of species, recognition of putative *Listeria* subtypes, and rapid discovery of previously unobserved operons or plasmids shared among *S. aureus* and other isolates. In the southwest Nigerian abattoirs, the same methodology interrogates mixed samples of carcass swabs and water for authentic profiling of meat-associated hazards, thus supplying counseled stakeholders, from regulators to operators.

Earlier investigations in Nigeria have documented that meat from abattoirs often harbours alarmingly elevated levels of bacteria, with some counts far exceeding globally accepted safety limits (Olaitan et al., 2025; Odetokun et al., 2023). Despite this, investigations at the level of individual species remain limited, obscuring a complete view of the microbial contaminants that may threaten human health. This research, therefore, aimed to fill the existing void by deploying 16S rRNA gene sequencing to molecularly profile bacteria from freshly slaughtered cow intestines acquired at the Ado-Ekiti abattoir. The specific aims of the project were: (i) to catalogue the bacterial species found in ready-to-eat cow intestine, (ii) to evaluate their prospective implications for public health, and (iii) to contextualize the observations against the wider body of literature relating to food safety and microbial hazards.

2. Materials and Methods

Sample Collection: Aseptic retrieval of freshly slaughtered cow intestines was carried out at the Ado-Ekiti abattoir. The procedure employed sterile gloves, forceps, and labelled containers in order to curtailed unwanted contamination. Collected intestines were sealed in sterile Whirl-Pak® bags, packaged in ice-cooled boxes to maintain the cold chain, and delivered to the laboratory for analysis within two hours after excision from the carcass.

Bacterial Isolation: The intestines were subsequently homogenized, and the resulting tissue emulsions were subjected to two-fold serial dilutions. These dilutions were inoculated on three solid media—MacConkey agar, nutrient agar, and selective media so as to obtain a complete profile of the microbial populations and promote the growth of diverse cultivable isolates for subsequent characterization. Distinct colonies were sub-cultured to obtain pure isolates, which were preserved in glycerol stocks at -20 °C until DNA extraction.

DNA Extraction: Genomic DNA was extracted using the Qiagen DNA mini kit following the manufacturer's protocol. Purity and concentration were verified with a Nanodrop spectrophotometer, and integrity was confirmed via 1% agarose gel electrophoresis.

PCR Amplification: The universal bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') were employed to amplify the 16S rRNA gene. Reactions were carried out in 25 µL volumes containing template DNA, Taq polymerase, primers, dNTPs, and buffer. The PCR cycling conditions included initial denaturation at 95 °C, followed by 30 cycles of denaturation, annealing, and extension, and a final elongation step.

Sequencing and Bioinformatics: Cleaned PCR products were sequenced by Sanger sequencing. Raw sequences were trimmed and ordered using the BioEdit software. BLAST comparisons against the NCBI GenBank database were done to locate closest relatives. Percent similarity cut-offs were utilized: ≥97% similarity was utilized as identification at species level, and lower similarity for possible novel or divergent strains.

Ethical Issues: Ethical clearance was obtained from the Federal Polytechnic Ethical Committee and due consent was given by the management of abattoir for sampling. The laboratory operations had precautions in accordance with biosafety guidelines to minimize exposure to pathogenic microbes.

3. Results

16S rRNA sequence data revealed eight isolates from seven bacterial species within distinct genera. Most of the isolates were highly similar (>95%) to reference organisms, demonstrating appropriate taxonomic resolution, but one isolate was low in similarity, suggesting potential novelty.

The following table shows the bacterial isolates, GenBank accession numbers, and percent similarities.

Table 1 16S rRNA gene sequencing showing different bacteria isolated from freshly killed ready-to-eat cow intestine from an abattoir in Ado-Ekiti, Nigeria

Code	Organism	GenBank Accession	% Similarity
BS3-3	*Providencia vermicola*	CP048796.1	97.74
S3-1	*Pseudomonas pseudoalcaligenes*	KF171340.1	99.93
BS6-3	*Comamonas testosterone*	MF993019.1	84.71
S6-1	*Escherichia coli*	MN208138.1	99.32
BS7-2	*Pseudomonas mendocina*	MT372152.1	99.77
S7-4	*Bacillus subtilis*	MK942526.1	99.78
BS9-1	*Providencia vermicola*	CP048796.1	95.98
S9-3	*Comamonas kerstersii*	CP020121.1	99.77

Among the identified species*, Providencia vermicola* appeared in two isolates, with sequence similarities of 97.74% and 95.98%, respectively. *Escherichia col*i, a canonical indicator of feacal contamination, showed a 99.32% similarity to reference sequences. *Pseudomonas* species were also well represented, with *Pseudomonas pseudoalcaligenes* and *Pseudomonas mendocina* showing very high similarity values (>99%). The *Bacillus subtilis* isolate demonstrated 99.78% similarity, confirming its ubiquity in soil and abattoir environments. Of particular interest was Comamonas testosterone, which showed only 84.71% similarity, raising the possibility of either misclassification or the presence of a novel strain in the abattoir environment.

4. Discussion

This work provides valuable molecular data on the bacterial load of freshly slaughtered cow intestines in the Ado-Ekiti abattoir. The variety of bacteria present indicates the complexity of microbial contamination pathways in meat processing (Gutema et al., 2021; Bedane et al., 2024; Uzoigwe et al., 2021). Several notable observations are marked in these results.

It is no surprise that *Escherichia coli* is found, as it is a documented faecal indicator organism (Gutema et al., 2021). *E. coli* is frequently reported in abattoir surveys all over the world and serves as an indicator of fecal contamination and poor hygienic practices (Gutema et al., 2021; Odetokun et al., 2023; Le et al., 2025). Pathogenic variants, such as enterohemorrhagic *E. coli* (EHEC), have been responsible for outbreaks of acute gastroenteritis and hemolytic uremic syndrome. Although this study did not subtype the isolates, the very high homology to reference sequences suggests the potential health issues implicated in the intake of contaminated intestine (Gutema et al., 2021; Bedane et al., 2024).

Providencia vermicola, twice isolated in this work, belongs to the Enterobacteriaceae and has been identified as a new opportunistic pathogen. *Providencia* infections have been reported in urinary tract infections, septicemia, and wound infections, especially, in immunocompromised patients, as clinical accounts.

The isolation of multiple Enterobacteriaceae, including *E. coli* in cow intestine samples confirmed the faecal route of contamination, signalling a potential reservoir for zoonotic pathogens (French et al., 2010). This reservoir is further compounded by the subsequent assortments of *Pseudomonas species*, notably *P. pseudoalcaligenes* and *P*. *mendocina*, frequently encountered in processes where fat and water mixtures co-habit the same surfaces. P. pseudoalcaligenes has been documented in sporadic but life-threatening patient profiles presenting with bacteremia and endocarditis, while the more the frequently recovered *P. mendocina* shows a further paradox wherein the organism has been associated with infections in immuno-compromised hosts (Paulsen et al., 2012). *Pseudomonas*' intrinsic and acquired resistances to multiple disinfectants and common therapeutic classes translate their recovery within abattoir microecosystems into a notable preventive concern for, organ-specific colonization that, once product packaged, presents further extension to the competitive microflora of human hosts. As such, a red flag in any of the duck, goat, or bovine flocks in which they were encountered translates into harvest and further rendering operational blight. Similarly, while the prior and widely employed industrial and probiotic applications of *Bacillus subtilis* suggest benign stewardship, sporulation remains aggrandized in the moist post-evisceration carnage. In such confined remains, the epiphytic assemblage of spores

enabled their prolonged survival and, amplified, the minority strains found to produce surfactant-like or enterotoxin-like moieties (sur c, cereulide, etc.) reinforce their prediction in cumulative load and post-hoc prophylaxis meta-analysis, regardless of the singular's original proximity to human virulence.

Comamonas testosterone, displaying a sequence similarity of 84.71%, warrants further scrutiny, as this deviation suggests the possible emergence of a neglected or poorly classified strain. Recent epidemiological literature has branded representatives of the genus *Comamonas* as potential opportunistic pathogens, a categorization corroborated by observed increases in bloodstream infections. Its appearance in meat of abattoir origin raises suspicions of horizontal environmental transfer, the clearest driver being contaminated aqueous resources governed by slaughter and processing. To provide taxonomic closure and virulence landscape definition, a proposal for supplementary Whole Genome Sequencing is unavoidable.

Comamonas kerstersii, with a clock-like 99.77% similarity, heightens the hypothesis of a *Comamonas*-associated food reservoir, a dogma that the scientific literature has understated. The literature raises the spectra of the organism in bloodstream pathologies, with reported roots in peritonitis and anonymized clinical bacteremia. Its reproducible presence in abattoir processing should prompt the embedding of routine microbial surveillance.

The aggregate observations corroborate narratives delivered by triad-based Nigerian datasets; thus, cross-continental reflection is possible. Studies have illustrated pervasive ready-to-eat bacteria contamination in meat from various states like Imo, Oyo, Osun and Lagos based on offal, mirroring chaotic ecologies (Uzoigwe et al., 2021; Ayoade et al., 2021; Olaitan et al., 2025). Parallel evidential loops have emerged from other parts of the world too (Le et al., 2025; Bhutia et al., 2021; Intongead et al., 2025). Lack of sewerage capacity and poor supply chain infrastructure remain prescient insulative drivers across the geography (Makinde et al., 2021; Intongead et al., 2025).

Beyond identification, public health repercussions are serious. Though prepared for eating, cow intestine is treated with only slight heat, attesting to intact pathogenic bacteria. Beyond acute symptoms, persistent bacteria may lead to prolonged cheat or even antimicrobial resistance (AMR). Abattoirs qualify easily as vectors for AMR (Olaitan et al., 2025; Odetokun et al., 2023). Antibiotics in feed and during treatment, sewn with slaughter confusion, allow resistant organisms to contaminate coherent meat surfaces and instruments. Routine culture of opportunistic bacteria such as *Pseudomonas* and *Providencia* increases peril, since both bacteria are experienced accumulators of fluctuating resistance and diverse resistance chromosomal cassettes.

Another pressing outcome attains degree in permissive nations regulation. Nationwide slaughter oversight suffers poverty of raw practical observation. Schemes to carcass examination, personnel hygiene, and hand washing reversion gather dust in well-printed folders, creating openings that slaughter hygiene education, sizable handset of technologists, and volunteering health surveillance finish, spill pathogens as happen. Trains run in Barshens and Dunn's investigative sequencers, could evolve an amendment organ, absorbing in their minds and describing well into treatment of tasks, help monthly report slaughter antimicrobial caliber national, secure in balance.

5. Conclusion

In summary, this investigation presents notable methodological strengths alongside inherent limitations. Although 16S rRNA sequencing successfully revealed microbial taxa that culture techniques frequently overlook, its inability to resolve closely related strains or link organisms to specific virulence profiles constrains its predictive power. Prospective work that pairs this tool with whole-genome sequencing, targeted metagenomic analysis, and antimicrobial susceptibility profiling would, however, paint a more complete picture of the microbial hazards linked to abattoir-derived meats.

The results compel immediate, concerted action at multiple levels: upgrading abattoir infrastructures, tightening hygiene compliance monitoring, training of slaughter personnel, and empowering consumers with ongoing education about the correct processing and cooking of offal. This study of cow intestines from the Ado-Ekiti abattoir has confirmed a diverse microbial community that includes, among others, pathogenic and potentially pathogenic species such as *Escherichia coli, Providencia vermicola, Pseudomonas pseudoalcaligenes, Pseudomonas mendocina, Bacillus subtilis,* and *Comamonas* species. Beyond the direct food-safety implications, the results underline pathways for zoonotic transmission and the wider, worrying amplitude of antimicrobial resistance, reinforcing the need for sustained and integrated control measures. The presence of a potentially novel *Comamonas* strain adds scientific value by expanding our understanding of microbial ecology in abattoir environments.

It is advised that governments enact cleanliness standards, improve abattoir facilities, and introduce molecular surveillance as part of standard monitoring in an attempt to safeguard public health. In a move to reduce dangers, consumers must be taught about handling and cooking cow intestine correctly. In the future, research should follow up on these findings using whole-genome sequencing and antimicrobial susceptibility profiling.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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