

Antibiotic resistance in ESKAPE bacteria group isolated from piggeries in Owo local government

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Abstract

The intensification of pig farming in Nigeria has led to increased antibiotic use, contributing to the emergence of antimicrobial resistance (AMR). This study assessed the antibiotic resistance profiles of ESKAPE bacteria isolated from piggery effluents in Owo Local Government. Effluent samples were collected from ten farm sites and analysed using microbiological and molecular techniques. Among the ESKAPE group, *Klebsiella* spp., *Pseudomonas* spp., *Escherichia coli*, and *Staphylococcus aureus* were identified. Antibiotic susceptibility testing revealed high resistance levels, with multiple isolates displaying elevated Multiple Antibiotic Resistance Index (MARI) values, indicating significant exposure to antimicrobials. Molecular characterization confirmed the presence of resistance-associated genes in *E. coli* and *S. aureus*, with over 99% sequence identity to previously reported resistant strains. The findings highlight the role of pig farming in the dissemination of multidrug-resistant bacteria, posing risks to public health through environmental contamination and potential zoonotic transmission. Urgent interventions, including stricter antibiotic regulations, enhanced surveillance, and antimicrobial stewardship, are recommended to mitigate the spread of resistant pathogens in livestock environments.

Keywords : Bacteria, Antibiotic resistance, piggery, effluent.

Résistance aux antibiotiques chez les bactéries du groupe ESKAPE isolées d'élevages porcins dans la zone de gouvernement local d'Owo

Résumé

L'intensification de l'élevage porcin au Nigéria a entraîné une augmentation de l'utilisation des antibiotiques, contribuant ainsi à l'émergence de la résistance aux antimicrobiens (RAM). Cette étude a évalué les profils de résistance aux antibiotiques des bactéries du groupe ESKAPE isolées à partir d'effluents d'élevages porcins dans la zone de gouvernement local d'Owo. Des échantillons d'effluents ont été prélevés sur dix sites d'élevage et analysés à l'aide de techniques microbiologiques et moléculaires. Parmi le groupe ESKAPE, *Klebsiella* spp., *Pseudomonas* spp., *Escherichia coli* et *Staphylococcus aureus* ont été identifiés. Les tests de sensibilité aux antibiotiques ont révélé des niveaux de résistance élevés, plusieurs isolats présentant des valeurs élevées de l'indice de multirésistance aux antibiotiques (MARI), ce qui indique une exposition significative aux antimicrobiens. La caractérisation moléculaire a confirmé la présence de gènes associés à la résistance chez *E. coli* et *S. aureus*, avec une identité de séquence supérieure à 99 % avec des souches résistantes précédemment rapportées. Ces résultats mettent en évidence le rôle de l'élevage porcin dans la dissémination de bactéries multirésistantes, ce qui pose des risques pour la santé publique en raison de la contamination de l'environnement et d'une potentielle transmission zoonotique. Des interventions urgentes, notamment des réglementations plus strictes sur les antibiotiques, une surveillance renforcée et une gestion responsable des antimicrobiens, sont recommandées pour atténuer la propagation de pathogènes résistants dans l'environnement de l'élevage.

Mots-clés : Bactéries, Résistance aux antibiotiques, Élevage porcin, Effluent.

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INTRODUCTION

In the last decade and a half, the Federal Government of Nigeria has been making concerted efforts to diversify the nation's economy away from oil dependence to an agriculture-driven one. This shift has led to a revolution in farming across the country, with significant increases in both crop and livestock production. Among the various livestock industries, pig farming has emerged as a critical sector, contributing substantially to Nigeria's protein supply chain. It ranks third after poultry and beef production, offering numerous advantages such as rapid growth rates, high reproductive efficiency, efficient feed conversion, and the ability to thrive in relatively small spaces (**Ezeibe, 2010**). These advantages, combined with the increasing demand for pork in the southern regions of the country, have driven significant growth in pig farming, primarily through intensive production systems.

However, the intensification of pig farming has inevitably led to the widespread and often indiscriminate use of antibiotics, both for disease prevention and as growth promoters (**Lekagul et al., 2020; Bennani et al., 2020**). This practice contributes to the emergence and dissemination of antibiotic-resistant bacteria, which pose serious threats to public health. The problem is exacerbated by inadequate regulatory enforcement and the ease of access to veterinary antibiotics, leading to their misuse and overuse in piggery operations (**Van Boeckel et al., 2015**).

The global antimicrobial consumption in livestock is predicted to increase by two-thirds over the next decade, with pig farming accounting for the highest usage compared to poultry and cattle (**Van Boeckel et al., 2015**). Antibiotics are frequently used in pig production to prevent infectious diseases, promote growth, and improve feed efficiency.

However, the routine use of these antimicrobials creates a strong selective pressure that fosters the survival and proliferation of antibiotic-resistant bacteria (**Liu et al., 2019**).

Despite growing concerns, monitoring efforts on antibiotic consumption and antimicrobial resistance (AMR) are still largely focused on clinical and public health laboratories, with limited attention given to the agricultural sector, particularly in low- and middle-income countries like Nigeria (**Abushaheen et al., 2020**). This gap in surveillance is particularly concerning given the potential for resistant bacteria from livestock to spread to humans through direct contact, consumption of contaminated meat, or environmental pathways such as effluent discharge into water bodies (**Brower et al., 2017**).

The ESKAPE group of bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) is of particular concern in antimicrobial resistance research. These bacteria are known for their ability to "escape" the effects of common antibiotics, contributing significantly to multidrug resistance in both human and veterinary medicine (Santajit and Indrawattana 2016). The presence of these pathogens in piggery effluent is alarming, as they can disseminate resistance genes into surrounding environments, leading to further spread in human and animal populations (**He et al., 2020**).

Recent studies have demonstrated that livestock-associated *S. aureus* (LA-MRSA) and multidrug-resistant *K. pneumoniae* are increasingly prevalent in pig farming environments, raising concerns about zoonotic

transmission and treatment failures in human medicine (McEwen and Collignon 2018). Furthermore, *P. aeruginosa* and *A. baumannii*, both notorious for their intrinsic resistance mechanisms, have been isolated from livestock settings, highlighting the role of agricultural practices in the evolution of drug-resistant infections (Schwarz *et al.*, 2018).

Given the increasing reliance on antibiotics in pig farming and the global rise of antimicrobial

resistance, there is an urgent need to assess the extent of antibiotic resistance in ESKAPE bacteria isolated from piggery effluents. Understanding the resistance profiles of these bacteria is crucial for informing antimicrobial stewardship policies, mitigating environmental contamination, and reducing the potential transfer of resistant strains to humans. Therefore, this study was conducted to determine the antibiotic resistance patterns of ESKAPE pathogens isolated from piggery effluent in Owo Local Government, Nigeria.

MATERIAL AND METHODS

1. Sampling

The 30 samples of piggery effluents were collected from 10 randomly selected farm sites in Owo local government using sterile and clean bottles.

2. Microbial analyses

The pour plate method was used for isolation of bacterial pathogens from all the samples using MacConkey agar. One milliliter of each sample was aseptically withdrawn with a sterile pipette and serially diluted in physiological saline to the fourth dilution using a ten-fold serial dilution. About 0.1 ml aliquot of each dilution was inoculated onto duplicate set of MacConkey agar aseptically. All the plates was incubated at 37°C for 24 hours.

Purification of isolate was done by randomly selecting single bacterial colony resulting from different culture plates based on their morphology and subsequently isolated in pure forms by sub-culturing on nutrient agar plates and stored on nutrient agar slants in the refrigerator at 4°C until used for microscopic characterization and biochemical analysis. Thereafter the identified isolate stocks was preserved at -80°C in nutrient broth containing (Salem *et al.*, 2024).

- Morphological and Biochemical Characterization of the Bacteria Isolates

The bacterial isolates was characterized and identified based on their microscopic morphology, colonial morphology and biochemical characterization as described in medical laboratory manual for tropical countries (Cheesbrough, 2005) and with reference to the Bergey's Manual of Systematic Bacteriology as described by Alagoa and Felagha, (2025)

3. Antibiotic susceptibility test and Determination of Multiple antibiotic resistance index (MARI)

The AST was carried out based on the CLSI guideline and MAR index for the resistant bacteria isolates was determined according to the procedure described by Law *et al.*, (2021). The formula used for calculation was

$$MARI = a \div b$$

Where a= number of antibiotics to which the isolate is resistant, b= total number of antibiotics used.

4. Molecular identification of isolate

DNA of isolates were extracted, amplified using PCR techniques followed by sequencing of the amplified fragments (He *et al.*, 2020).

4.1. Bacteria DNA extraction

DNA was extracted using the protocol stated by (1). Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28 °C. After this period, cultures were centrifuged at 4600g for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37 °C, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and vortexed. The suspension was incubated for 10 min at 65 °C and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200 g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA

precipitated at -20°C for 16 h. DNA was collected by centrifugation at 13000 *g* for 10 min, washed with 500 μl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 μl of TE buffer.

4.2. PCR Analysis

PCR sequencing preparation cocktail consisted of 10 μl of 5x GoTaq colourless reaction, 3 μl of 25mM MgCl_2 , 1 μl of 10 mM of dNTPs mix, 1 μl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5'- AAGGAGGTGATCCAGCC-3' primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 μl with sterile distilled water 8 μl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 50°C for 60s and 72°C for 1 minute 30 seconds ; and a final termination at 72°C for 10 mins. And chill at 4°C .GEL (2,3)

4.3. INTEGRITY

The integrity of the amplified gene fragment was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3 μl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gives color and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4 μl of each PCR product and loaded into the wells after the 100bp

DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

4.4. PURIFICATION OF AMPLIFIED PRODUCT

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 μl of Na acetate 3M and 240 μl of 95% ethanol were added to each about 40 μl PCR amplified product in a new sterile 1.5 μl tube eppendorf, mix thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for 10 min at 13000 *g* and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 μl of 70% ethanol and mix then centrifuge for 15 min at 7500 *g* and 4°C . Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then resuspend with 20 μl of sterile distilled water and kept in -20°C prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified productand quantified using a nanodrop of model 2000 from thermo scientific.

4.5. SEQUENCING

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis

BLASTN search was carried out to determine the identity of the selected isolates.

RESULTS AND DISCUSSION

1. Occurrence of ESKAPE Bacteria in Piggery Effluent

The present study investigated the occurrence of ESKAPE bacteria in piggery effluent and identified four members of this clinically significant group: *Klebsiella* spp., *Pseudomonas* spp., *Escherichia coli*, and *Staphylococcus aureus*. Although the classical ESKAPE group includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp., only a subset was recovered in this study. The absence of *Enterococcus* spp., *Acinetobacter* spp., and *Enterobacter* spp. may reflect environmental and selective pressures within the piggery ecosystem.

One plausible explanation for the limited recovery of the full ESKAPE spectrum is the intensive and often indiscriminate use of antibiotics in pig production systems. In many commercial piggeries, antibiotics are routinely administered not

only for therapeutic purposes but also for prophylaxis and growth promotion. Such sustained antimicrobial pressure may suppress susceptible bacterial populations while selectively enriching resistant strains capable of surviving in antibiotic-laden environments. Consequently, certain bacterial species may be attenuated or eliminated, reducing their detectability in effluent samples.

The detection of *Klebsiella* spp., *Pseudomonas* spp., *E. coli*, and *S. aureus* is epidemiologically significant. These organisms are well-recognized opportunistic pathogens implicated in both veterinary and human infections. Their presence in piggery effluent highlights the farm environment as a potential reservoir and amplification point for antimicrobial resistance (AMR). Similar findings have been reported in livestock-associated studies, where ESKAPE pathogens were frequently isolated from manure, wastewater, and farm surfaces (Bennani et al., 2020; Abushaheen et al., 2020). (Table 01)

Table 1: Biochemical characteristics of the isolates

Probable organism	BIOCHEMICAL TEST RESULTS										
	Gram Rxn	OXI	COAG	CAT	URE ASE	MO TIL ITY	CIT	GLU	LAC TOS E	MAL TOS E	IND OLE
<i>Klebsiella</i> spp	-	-	-	+	+	-	+	+	+	+	-
<i>Pseudomonas</i> spp	-	+	-	+	-	+	-	-	+	-	-
<i>Staphylococcus</i> spp	+	-	+	+	-	-	-	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-	-	+	-	+	+	-	+

Key: - = negative, + = Positive

Escherichia coli, commonly found in the intestinal tract of animals, serves as an important indicator organism for fecal contamination and antimicrobial resistance surveillance. Its detection in effluent suggests the potential dissemination of resistant strains into surrounding soil and aquatic environments. Likewise, *Staphylococcus aureus*, particularly livestock-associated strains, has been increasingly associated with zoonotic transmission and community-acquired infections. The detection of these organisms in agricultural waste underscores the interconnectedness of animal health, environmental health, and human health within the One Health framework.

Environmental persistence further compounds the risk. Members of *Pseudomonas* and *Klebsiella* genera possess adaptive traits such as biofilm formation, efflux pumps, and enzymatic degradation of antimicrobials, enhancing their survival in harsh environmental conditions. Effluent discharge into surface water bodies may therefore serve as a conduit for resistant bacteria to enter broader ecological systems, including irrigation water, crops, and drinking water sources.

The ecological interface between livestock production and the environment facilitates microbial exchange. In settings where piggery effluent is inadequately treated before disposal, resistant bacteria and resistance genes may accumulate in

sediments, soils, and aquatic ecosystems. Studies have demonstrated that agricultural runoff significantly contributes to the environmental resistome, increasing the background levels of resistance genes in natural microbial communities (Zhang *et al.*, 2021). Thus, the occurrence of ESKAPE organisms in piggery effluent is not merely a localized farm issue but a public health concern with far-reaching implications.

2. Antibiotic Resistance pattern of the isolates

The antibiotic susceptibility testing revealed that two isolates exhibited particularly high resistance levels, as evidenced by elevated Multiple Antibiotic Resistance Index (MARI) scores. High MARI values (>0.2) typically indicate that bacterial isolates originate from high-risk sources with frequent antibiotic exposure. In this context, the results strongly suggest sustained antimicrobial selection pressure within the piggery environment (Table 02, Table 03).

Table 2: Antibiotics susceptibility profile of the isolated *E. coli*

Isolate code	ANTIBIOTICS												MAR index
	AUG 30µg	CTX 25µg	IMP 10µg	OFX 5µg	GN 10µg	NA 30µg	NF 300µg	CXM 30µg	CRO 45µg	ACX 10µg	ZEM 5µg	LBC 5µg	
AI	R	R	R	S	R	R	R	R	R	R	R	S	0.84
CI	R	R	R	S	R	R	R	R	R	R	R	S	0.84
FII	R	R	S	S	R	R	R	R	R	R	R	S	0.75
HI	R	R	R	S	R	R	R	R	R	R	R	S	0.84
JIV	S	R	R	S	R	R	R	S	R	R	R	S	0.66

Key: **IMP:**Imipenen/cilastain, **OFX:**Ofloxacin, **GN:**Gentamycin, **NA:** Nalidixic Acid, **NF:**Nitroflurantoine, **CXM:**Cefuroxime, **CRO:**Ceftriaxone Sulbactain, **ACX:**Ampliclox, **ZEM:**Cefexine, **LBC:** Levofloxacin, **AZN:**Azithromycin, **ERY:**Erythromycin, **CIP:**Ciprofloxacin

Table 3: Antibiotics susceptibility profile of the isolated *Staphylococcus aureus*

Isolate code	ANTIBIOTICS												MAR index
	AUG 30µg	CTX 25µg	IMP 10µg	OFX 5µg	GN 10µg	CIP 5µg	ERY 15µg	CXM 30µg	CRO 45µg	AZN 15µg	ZEM 5µg	LBC 5µg	
AI	R	R	S	S	S	S	S	R	R	S	R	S	0.42
EI	R	S	S	S	S	S	S	R	R	S	R	S	0.33
FI	R	R	S	S	S	S	S	R	R	S	R	S	0.42
GII	R	R	S	S	S	S	S	R	R	R	R	S	0.50
JI	R	R	S	S	S	S	S	R	R	S	R	S	0.42

Key: **VIIMP:**Imipenen/cilastain, **OFX:**Ofloxacin, **GN:**Gentamycin, **NA:** Nalidixic Acid, **NF:**Nitroflurantoine, **CXM:**Cefuroxime, **CRO:**Ceftriaxone Sulbactain, **ACX:**Ampliclox, **ZEM:**Cefexine, **LBC:** Levofloxacin, **AZN:**Azithromycin, **ERY:**Erythromycin, **CIP:**Ciprofloxacin

The observed resistance patterns likely reflect indiscriminate antibiotic usage practices, including subtherapeutic dosing and prolonged prophylactic administration. Such practices create ideal

conditions for the selection of multidrug-resistant (MDR) strains. Van Boeckel *et al.*, (2015) projected that antimicrobial consumption in livestock would rise substantially, with pigs representing one of the

highest-consuming sectors globally. This trend has continued, particularly in low- and middle-income countries where regulatory enforcement may be limited.

The broad-spectrum resistance observed among isolates in this study suggests the presence of both intrinsic and acquired resistance mechanisms. Intrinsic resistance may arise from structural or physiological features such as reduced membrane permeability or chromosomally encoded efflux pumps. However, acquired resistance, often mediated by plasmids, transposons, and integrons, poses a greater epidemiological threat due to its transmissibility across bacterial species.

Staphylococcus aureus is particularly notable for its genetic plasticity and ability to acquire resistance determinants, including *mecA*-mediated methicillin resistance and genes conferring resistance to macrolides, aminoglycosides, and fluoroquinolones. Similarly, *E. coli* and *Klebsiella spp.* are known reservoirs of extended-spectrum beta-lactamases (ESBLs) and carbapenemases. The presence of such organisms in agricultural effluent suggests that farm environments may act as silent reservoirs of clinically relevant resistance genes.

Horizontal gene transfer (HGT) plays a central role in the dissemination of resistance. Conjugative

3. Molecular Identity of the isolates

Molecular characterization through 16S rRNA gene amplification confirmed the identity of two highly resistant isolates as *Escherichia coli* and

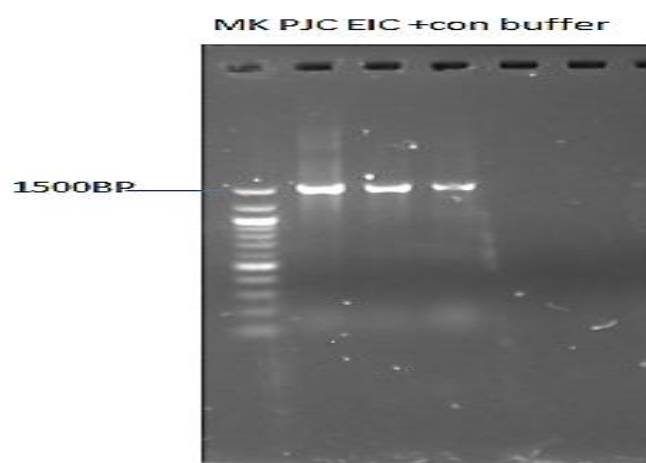
plasmids can transfer resistance determinants among coexisting microbial populations within manure lagoons and wastewater systems. Integrons further enhance adaptability by capturing gene cassettes encoding resistance traits. Johnson *et al.*, (2016) demonstrated that agricultural effluent environments facilitate genetic exchange due to high microbial density and nutrient availability.

The environmental release of resistant bacteria through effluent discharge introduces resistance genes into natural microbial communities. Once in the environment, these genes can be incorporated into commensal bacteria or opportunistic pathogens, increasing the risk of treatment failures in clinical settings. Ma *et al.*, (2014) highlighted that resistance genes originating from animal production systems have been detected in downstream water bodies used for human consumption and irrigation.

The public health implications are substantial. Communities relying on untreated surface water for domestic use may be exposed to resistant pathogens. Furthermore, crops irrigated with contaminated water can act as secondary transmission pathways. The convergence of environmental contamination and human exposure exemplifies the urgency of strengthening antibiotic stewardship in agricultural settings.

Staphylococcus aureus, with sequence identities of 99.49% and 99.42%, respectively. The approximately 1500 bp amplicons correspond to the conserved 16S rRNA gene region widely used for bacterial taxonomic identification (Figure 1).

Figure 1: Agarose Gel showing the positive amplification of the 16S regions amplified from the selected bacteria samples.



High sequence similarity to previously reported strains suggests phylogenetic relatedness to established lineages. However, minor nucleotide variations may indicate evolutionary divergence or adaptation to local selective pressures. Such variations may reflect the acquisition of mobile genetic elements carrying resistance determinants.

The close genetic resemblance to documented resistant strains (**He et al., 2020**) supports the hypothesis that these isolates may harbor transferable resistance genes acquired through horizontal gene transfer. Mechanisms such as plasmid exchange, transformation, and bacteriophage-mediated transduction enable rapid dissemination of resistance traits in microbial communities subjected to antibiotic stress (**Law et al., 2021**).

The cohabitation of multiple resistant species within the same ecological niche increases the likelihood of plasmid exchange. Piggery effluent represents a nutrient-rich, densely populated microbial environment conducive to genetic recombination. This environment may facilitate the emergence of novel resistance combinations, potentially reducing the efficacy of last-resort antibiotics such as carbapenems and fluoroquinolones.

Comparative studies have demonstrated similarities between resistance profiles in livestock-

associated bacteria and clinical isolates. **Rosengren et al., (2013)** reported that *E. coli* isolates from pigs harbored resistance genes identical to those found in human pathogens. Similarly, **Lekagul et al., (2020)** documented widespread ESBL-producing Enterobacteriaceae in pig farming environments in Southeast Asia, underscoring the global dimension of livestock-associated AMR.

The identification of variants closely related to known strains suggests ongoing evolution under antimicrobial pressure. Continuous exposure to sublethal antibiotic concentrations may promote mutational events and selection of adaptive phenotypes. Over time, such selective forces may contribute to the establishment of farm-specific resistant clones capable of persisting in both environmental and clinical settings.

The findings of this study reinforce the One Health concept, emphasizing the interconnectedness of human, animal, and environmental health. Piggery effluent serves as a critical interface for the dissemination of antimicrobial resistance. Without effective wastewater treatment and antibiotic stewardship policies, resistant bacteria may spread beyond farm boundaries into surrounding ecosystems

CONCLUSIONS

Based on the results obtained in this study, it is may be concluded that the isolates from piggery effluent are highly resistant to the commercially available antibiotics. Despite the need for further confirmatory studies, it is strongly recommended that campaigns on the discouragement of indiscriminate antibiotics use among pig farmers should be embarked on by the government.

This study underscores the critical role of intensive pig farming in the emergence of antibiotic-

resistant bacteria. The high MAR index values observed indicate a significant risk of resistance dissemination within and beyond piggery environments. Immediate interventions are needed to mitigate this threat, including policy reforms, enhanced monitoring, and the promotion of sustainable farming practices. Further research should focus on characterizing resistance genes at a genomic level to better understand their transmission dynamics and inform targeted mitigation strategies

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