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Pilot Passive AMR Surveillance in Diseased Terrestrial Food Producing Animals

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DISCLAIMER STATEMENT ON THE PRELIMINARY NATURE AND USE OF THIS REPORT

This document presents findings from the **"Pilot Passive AMR Surveillance in Diseased Terrestrial Food Producing Animals"** conducted from 15th September 2024 to 15th September 2025.

It is crucial to note that:

1. This was a **pilot study** designed to establish methods, protocols, build capacity, and generate initial baseline data.
2. The findings are based on a **limited sample size** (from 14 sentinel sites) and are **not representative** of all diseased food animals or species in Pakistan.
3. The antimicrobial susceptibility testing was performed using phenotypic methods only. **Absence of molecular confirmation** (e.g., PCR for resistance genes) means the genetic mechanisms of resistance are not characterized in this report.

Therefore, the data and conclusions in this report are strictly preliminary. They are intended for:

- Informing the design of future, more robust surveillance studies in diseased food producing animals.
- Guiding stakeholder discussions on AMR in the One Health context.
- Strengthening national technical capacity.

This report and its data must not be used for:

- Making export-related decisions or certifications.
- Enacting immediate regulatory measures against the animal health sector.
- Drawing definitive national-level estimates of AMR prevalence.

Foreword

Livestock and poultry are central to national food security, rural livelihoods, and economic stability, providing affordable sources of high-quality animal protein and supporting millions of farming households. The health and productivity of these sectors are therefore critical to sustaining food systems and safeguarding public health. However, the widespread and often unregulated use of antimicrobials in food-producing animals has contributed to the emergence and spread of antimicrobial resistance (AMR), posing a serious threat to animal health, food safety, and human well-being under the One Health framework.

Recognizing the importance of addressing AMR in livestock and poultry, the Government has prioritized the establishment of robust AMR monitoring and surveillance systems. Passive AMR surveillance, based on routine diagnostic submissions from diseased animals, represents a practical and sustainable approach to generating nationally relevant AMR data. In this context, the Ministry of National Food Security and Research (MoNFS&R) has implemented the "National AMR Surveillance Strategy for Diseased Food-Producing Animals," with an emphasis on passive surveillance in livestock and poultry sectors. Through standardized laboratory protocols, coordinated data collection, and centralized analysis, this system enables the identification of resistance trends and supports evidence-based decision-making for antimicrobial stewardship and disease control.

The Government remains firmly committed to sustaining and strengthening passive AMR surveillance in livestock and poultry across the country. Continued investment in laboratory capacity, workforce development, and data management systems is essential to ensure the long-term functionality and impact of these efforts. By maintaining strong collaboration among federal and provincial institutions and aligning surveillance outputs with national AMR action plans, Pakistan aims to protect animal health, enhance productivity, and reduce the risk of AMR transmission along the animal–human–environment interface. Sustained AMR surveillance in livestock and poultry is therefore a cornerstone of the national response to antimicrobial resistance and a critical investment in the country's health and food security future.

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Message from Team Lead, Fleming Fund Country Grant Pakistan (DAI)

The use of antimicrobials in livestock and poultry production systems has contributed significantly to improvements in animal health and productivity; however, inappropriate and excessive use may accelerate the emergence and spread of AMR, with serious implications for animal health, food safety, and public health. In this context, the timely detection of antimicrobial-resistant pathogens through passive AMR surveillance—based on routine diagnostic submissions from diseased animals—is essential for understanding resistance trends and guiding responsible antimicrobial use. This document provides a structured framework to support national authorities in strengthening passive surveillance systems and implementing evidence-based interventions to mitigate AMR risks in livestock and poultry sectors.

Antimicrobial resistance is a growing global concern that requires coordinated and sustained action. Recognizing the complex and interconnected nature of AMR, national governments and international partners have increasingly adopted a One Health approach that integrates human, animal, and environmental health considerations. Passive AMR surveillance in food-producing animals represents a practical and sustainable mechanism for generating critical resistance data, particularly in resource-constrained settings, and plays a vital role in informing antimicrobial stewardship, disease control strategies, and policy development.

The Fleming Fund, established by the United Kingdom in response to the UK AMR Review and the WHO Global Action Plan on AMR, has supported Pakistan since 2019 in strengthening AMR surveillance and laboratory systems across the human, veterinary, and environmental sectors. As part of this collaboration, significant progress has been made in establishing passive AMR surveillance in livestock and poultry through standardized diagnostic protocols, coordinated data collection, and centralized analysis. With the completion of the Fleming Fund Country Grant Pakistan (FFCGP), it is hoped that the Government of Pakistan (GoP) will continue to sustain and build upon these achievements. Continued leadership, institutional ownership, and investment will be essential to ensure the long-term functionality of passive AMR surveillance systems and to safeguard animal health, food security, and public health under the One Health framework.

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Contents

List of Acronyms

AHC	Animal Husbandry Commissioner
AMR	Antimicrobial Resistance
AMR-CU	AMR Coordination Unit
AMS	Antimicrobial Stewardship
AMU	Antimicrobial Use
ARG	Antimicrobial Resistant Genes
ARB	Antimicrobial Resistant Bacteria
AST	Antimicrobial Susceptibility Testing
CIA	Critically Important Antibiotics
CLSI	Clinical and Laboratory Standards Institute
FFCGP	Fleming Fund Country Grant, Pakistan
FPA	Food Producing Animals
GoP	Government of Pakistan
HIA	Highly Important Antibiotics
HPCIA	Highest Priority Critically Important Antibiotics
KPK	Khyber Pakhtunkhwa
MIA	Medically Important Antibiotics
MDR	Multi Drug Resistance
MoNFS&R	Ministry of National Food Security & Research
NRLPD	National Reference Laboratory for Poultry Diseases
NVL	National Veterinary Laboratories
SDG	Sustainable Development Goals
TSI	Triple Sugar Iron
TWG	Technical Working Group
WOAH	World Organization of Animal Health
WHO	World Health Organisation
XDR	Extensively drug resistant

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Executive Summary

This report presents the findings of Pakistan's first nationally coordinated passive antimicrobial resistance (AMR) surveillance programme in diseased food-producing animals (FPAs), implemented under Phase II of the Fleming Fund Country Grant to Pakistan (FFCGP). Between September 2024 and September 2025, a total of 11,915 diagnostic samples from poultry (broilers/layer and their breeder) and livestock including small ruminants (SR) and large ruminants (LR) were analysed through a newly established national network of veterinary diagnostic laboratories, generating the first baseline AMR evidence from diseased food producing terrestrial animals.

The surveillance network comprised 14 sentinel laboratories across federal and provincial public laboratories and major private veterinary diagnostic facilities. This public–private laboratory network, coordinated by the AMR Coordination Unit (AMR-CU) at the Ministry of National Food Security and Research (MoNFS&R), demonstrated the feasibility of routine, standardized data generation from clinical animal health submissions. Beyond producing AMR data, the programme successfully operationalised a functional veterinary diagnostic and reporting network, establishing a platform for future national AMR surveillance in diseased animals.

Key Findings

- **Widespread multidrug resistance (MDR)**: MDR was highly prevalent in poultry (*E. coli* 78.8%, *Salmonella* spp. 70.0%, *S. aureus* 63.8%) and substantial in livestock, including *E. coli* (LR 46.4%, SR 28.6%), *Salmonella* spp. (LR 58.8%, SR 23.8%), and *S. aureus* (LR 41.4%, SR 35.7%). This is likely to seriously compromise the effectiveness of antimicrobials for treating diseases associated with bacterial pathogens in poultry, LR and SR.

- **High resistance in *E. coli* to critically important antimicrobials**: *E. coli* showed very high resistance to fluoroquinolones (categorized as Highest Priority Critically Important Antimicrobials [HPCIA] by the World Health Organization), including enrofloxacin, a veterinary fluoroquinolone (poultry 89.9%; LR 89.4%; SR 100%) and norfloxacin (poultry 74.8%), with consistently high resistance to CIA agents such as streptomycin across animal groups.

- **Fluoroquinolone resistance in *Salmonella* across animal hosts**: *Salmonella* spp. exhibited high resistance to enrofloxacin in poultry (81.9%) and large ruminants (71.4%), and moderate to high resistance in small ruminants (50.0%), indicating reduced effectiveness of this key antimicrobial class.

- **Elevated resistance in *S. aureus* to priority antimicrobials**: *S. aureus* demonstrated high resistance to fluoroquinolones across all animal groups, with additional moderate to high resistance to cephalosporins (cefoxitin).

- **System-wide AMR occurrence:** AMR was evident across both poultry and livestock sectors, confirming that resistance is not sector-specific and highlighting the need for coordinated, sector-wide antimicrobial stewardship (AMS) and sustained national AMR surveillance complemented by a comprehensive antimicrobial use (AMU) and sales/distribution monitoring.

The integration of detailed metadata (specimen-origin) data enhances interpretability by linking resistance patterns to specific disease syndromes, such as mastitis and systemic infections, which impose significant economic and productivity losses in ruminants and poultry species. This emphasises the value of passive surveillance, supporting targeted interventions in animal health management, AMS, and disease control.

Looking forward, the FFCGP-supported programme demonstrates a feasible mechanism for establishing a sustainable national AMR surveillance system for diseased terrestrial animals beyond the Grant. The participating laboratories are well positioned to continue data generation using existing infrastructure and human resources. However, long-term sustainability will require continued government ownership, dedicated budgetary support, and further strengthening of harmonised laboratory methods (specifically the AST panel), quality assurance systems (integrating quality systems in private laboratories), and standardised data management. Priority areas for improvement include harmonisation of AST panels, routine reporting of both quantitative and raw AST results and continued training.

In conclusion, this initiative delivers both foundational AMR evidence and a functional national surveillance architecture for diseased food-producing animals in Pakistan. With targeted investments and sustained institutional commitment, this system can become a cornerstone of Pakistan's national AMR containment strategy by improving animal health outcomes, strengthening AMS, and contributing meaningfully to the One Health response to AMR.

1 Introduction

1.1 Background

Antimicrobial Resistance (AMR) is a major public health challenge driven by the emergence, spread and persistence of antimicrobial resistant microorganisms across humans, animals, and the environment. In 2019, AMR was estimated to have directly caused 1.27 million deaths worldwide[1], with projections indicating this burden could rise to 10 million deaths annually by 2050[2]. Beyond its direct health impacts, AMR threatens progress toward nearly all Sustainable Development Goals (SDGs), particularly those related to poverty, hunger, health, and economic growth[3,4]. Antimicrobials are widely used in food-producing animals for both therapeutic and disease prevention/control purposes. Consequently, meat production is projected to rise from 200 million tonnes to 470 million tonnes by 2050[5], intensifying the need to preserve the remaining antimicrobials through AMS in the animal sector.

The livestock and poultry sectors are recognized as important reservoirs of antimicrobial-resistant bacteria due to the widespread use of antimicrobials. Resistant bacteria originating in animals can spread to humans through direct contact, the food chain, or the environment, highlighting the interconnectedness of animal and public health under the One Health framework. Typically, the surveillance of diseased terrestrial and aquatic animals is implemented as passive surveillance programme, this surveillance relies on existing infrastructure such as veterinary diagnostic submissions or field investigations. Under the Food and Agriculture Organizations' International FAO Antimicrobial Resistance Monitoring (InFARM) system has a prevailing purpose of informing clinical decisions and antimicrobial treatment effectiveness for protecting animal health. Passive surveillance of AMR may provide additional information for protecting both animal and public health[6].

As livestock production expands, farmers may increasingly rely on antimicrobials to treat, prevent, and control infectious diseases in animals. The use of antimicrobials in food-producing animals has drawn substantial attention, as it contributes to the emergence, maintenance, and spread of AMR organisms, including those of significant public health relevance. This ecological dynamic underscores AMR as a critical One-Health challenge at the human-animal-environment interface. Transmission to humans occur primarily through the consumption of contaminated meat [2, 3], as well through direct or indirect contact with infected animals, animal wastes/manure, or contaminated environment[2].

Under Phase I of FFCGP, a National AMR surveillance strategy for both healthy and diseased FPAs. Two laboratories, the National Reference Laboratory for Poultry Diseases (NRLPD) and National Veterinary Laboratory (NVL), were formally designated as National Reference Laboratories (NRLs) for AMR surveillance in the animal health sector. During FFCGP Phase I these laboratories implemented active AMR surveillance in healthy FPA, generating baseline national AMR data. The core functions of the

[1]Chaudhry S, 2022. India's AMR burden has reached alarming proportions. Available from: http://bwhealthcareworld.businessworld.in/article/India-s-AMR-Burden-Has-Reached-Alarming-Proportions-Saransh-Chaudhary-/23-06-2022-433804/.

[2]Tackling drug-resistant infections globally: final report and recommendations. London: The Review on Antimicrobial Resistance; 2016. Available from: https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf.

[3]Paphitou NI, 2013. Antimicrobial resistance: action to combat the rising microbial challenges. Int J Antimicrob Agents, 42 Suppl: S25–28.

[4]Mamun M, Hassan J, Nazir K, Islam A, Zesmin K, Rahman B, 2017. Prevalence and molecular detection of quinolone-resistant *E. coli* in rectal swab of apparently healthy cattle in Bangladesh. Int J Trop Dis Health, 24:1–7.

[5]2050: A third more mouths to feed. Rome: Food and Agriculture Organization of the United Nations; 2009. Available from: http://www.fao. org/news/story/en/item/35571/icode/

[6] WOAH (formerly OIE). Terrestrial Animal Health Code.Chapter 6.8 Harmonisation of national antimicrobial resistance surveillance and monitoring programmes.

NRL's include providing technical leadership for AMR surveillance in animals, harmonising laboratory methodologies according to international guidelines recommended by FAO, WOAH and Codex, ensuring the quality and comparability of the data, and facilitating national AMR data reporting and communication, including routinely contributing to global surveillance platform such as FAO's InFARM.

Strengthening passive AMR surveillance systems in poultry and livestock supports evidence-based policymaking by generating nationally representative AMR that can guide AMS interventions. When coordinated through central AMR units and standardized veterinary diagnostic protocols (i.e., from post-mortem sample collection to microbial detection and antimicrobial susceptibility testing [AST]), such surveillance enhances data comparability and reliability while optimising existing resources and infrastructure. Integrating passive surveillance findings into national AMR action plans is essential for monitoring progress, optimising AMU, and safeguarding the long-term sustainability of livestock production systems[7]. Therefore, FFCGP Phase II was initiated in January 2024 with a focus on strengthening AMR surveillance systems in the livestock and poultry sectors. Under this initiative, the MoNFS&R was supported in the implementation of the National AMR Surveillance Strategy for Diseased FPAs, with particular emphasis on passive AMR surveillance. In this framework, the AMR Coordination Unit (AMR-CU) at MoNFS&R was designated as the lead entity responsible for the systematic collection, collation, and analysis of AMR data generated from livestock and poultry disease investigations. This approach has significantly enhanced the country's capacity to monitor resistance trends, generate evidence-based insights, and inform policy decisions aimed at mitigating AMR in food-producing animal systems.

1.2 Passive AMR Surveillance in Diseased Food Producing Animal

This report presents the methodology, findings, and implications from the pioneering passive AMR surveillance in diseased FPAs. To anchor this initiative within a robust national framework, the MoNFS&R took concrete institutional steps. Operational leadership for data collection, collation, and analysis was assigned to the Ministry's AMR-Coordination Unit (AMR-CU).

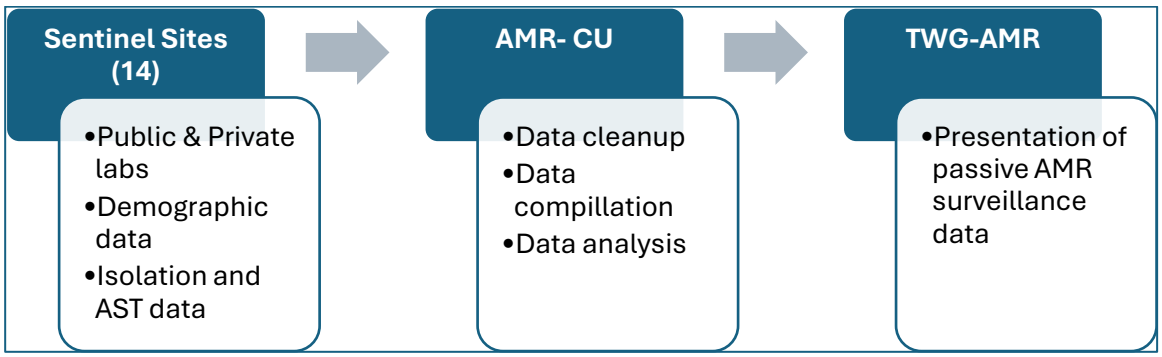

Figure 1: The process chart shows the data flow from sentinel sites to AMR-CU, ultimately presented at TWG for AMR. A network of 14 sentinel sites was developed that included laboratories from public and private sector. These laboratories shared AMR data with AMR-CU. At the AMR-CU the data was cleaned, compiled and analysed quarterly. The results were presented at TWG forum.

Furthermore, FFCGP championed a transformative public-private alliance by facilitating a Memorandum of Understanding (MoU) between private disease diagnostic laboratories as sentinel sites and DAI, the FFCGP implementation lead in Pakistan, with the MoNFS&R

[7] FAO. Antimicrobial Resistance Policy Review and Surveillance Guidance. 2021.

officially witnessing the agreement. This partnership is a critical step towards a coordinated sector-wide approach to AMR surveillance.

Figure 2: CEO's and representatives of five private stakeholders – (LEFT to RIGHT) Dr. Aleem Bhatti (GP Labs), Dr. Kashif Saleemi (Mukhtar Labs), AHC (MoNFS&R), Mr. Khalil Sattar (K&Ns), Dr. Qadeer Ahsan (DAI-Fleming Fund Country Grant Pakistan), Dr. Khalid Naeem (Averroes labs) signing the Memorandum of Understanding (MoU) with DAI-Pakistan and MoNFS&R (as witness) for sharing passive AMR surveillance data.

1.3 Aims and objectives

The aim of this pilot surveillance was to initiate and integrate passive AMR surveillance within Pakistan's animal health sector, in alignment with the National Action Plan on AMR. After piloting an active national AMR surveillance in healthy food animals (Year 2021-2022) to estimate potential public heath AMR risks associated with enteric bacteria carried by healthy animals, the current pilot surveillance sought to establish foundational systems and capacities needed to support passive surveillance to estimate AMR in *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* isolated from diseased food animals to inform treatment options for these diseases..

The objectives were two-fold:

- To strengthen national capacity across both public and private sector animal health stakeholders for implementing passive AMR surveillance, including bacterial isolation, AST, compliance with international AST guidelines, standardized reporting methodology, and analysis.
- To characterize and compare the AMR phenotypes of, *E. coli*, *Salmonella* spp. and *Staphylococcus aureus* recovered from large ruminants (cattle and buffalo), small ruminants (sheep and goat) and poultry, including the proportion of multidrug resistance (MDR) and resistance to WHO's medically important antimicrobials (MIA) for human medicine.

2 Methodology

2.1 Sampling sites

Under the auspices of the MoNFS&R, the FFCGP Phase II project established a national passive surveillance network for AMR. The network consisted of fourteen sentinel sites that voluntarily submitted laboratory data to the AMR-CU. As indicated in the accompanying map below (Figure 3), this represented a strategic public-private partnership, comprising eight public-sector disease diagnostic laboratories and six private facilities. The private laboratories were operated by Pakistan's foremost poultry integrators, namely K&N, Sadiq Brothers (SB Labs), and Big Bird (GP Labs) (Table 1).

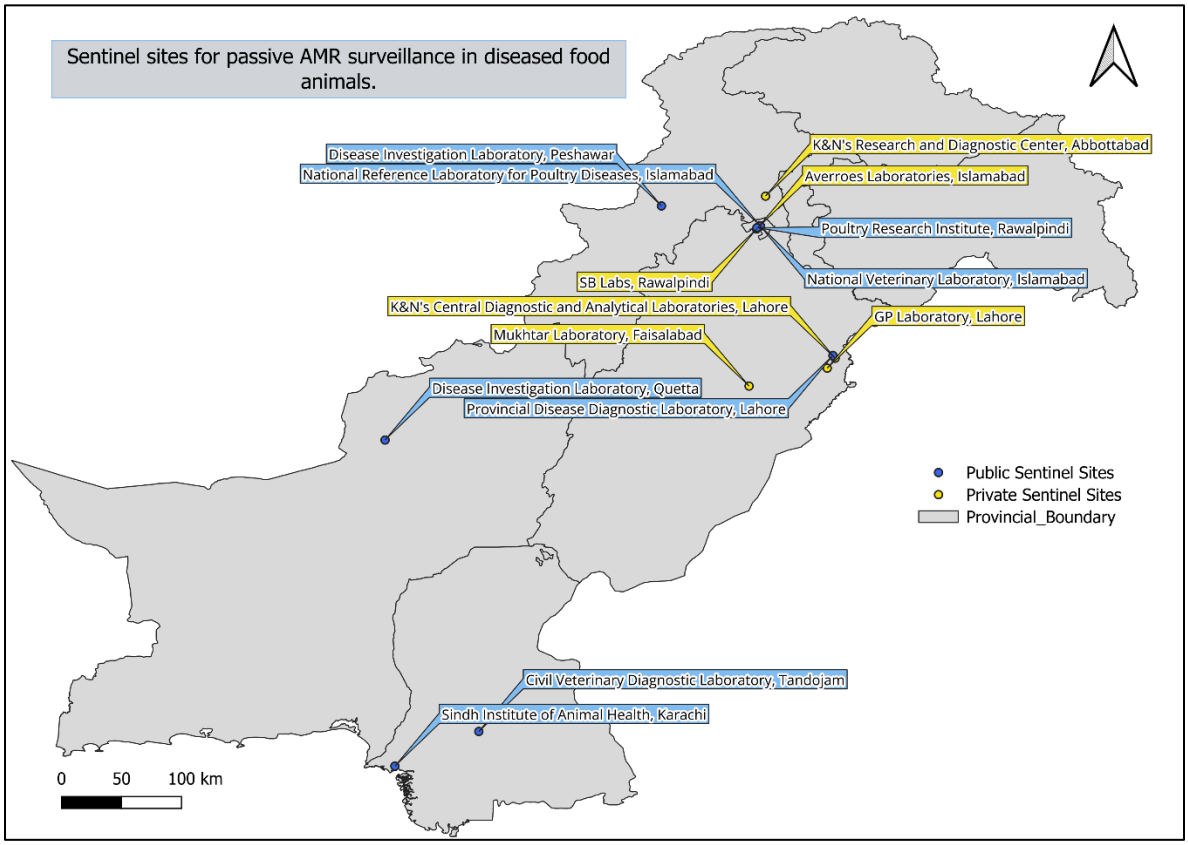

Figure 3: The map of Pakistan shows the fourteen (n = 14) sentinel sites for passive AMR surveillance. The point map shows inclusion of labs from major livestock and poultry producing areas of Pakistan.

To formalise the collaboration and ensure its continuity, a Memorandum of Understanding (MoU) was executed between the participating private laboratories and DAI Pakistan, with the MoNFS&R serving as the official witness. This agreement was pivotal in institutionalising the alliance between the government and private sector for AMR surveillance. The establishment of this coordinated network constitutes the first documented instance of a structured public-private partnership for passive AMR surveillance in animal health sector Pakistan.

Table 1: List of sentinel sites including public and private sector labs enrolled in the passive AMR surveillance network in diseased food producing animals.

Sr. No.	Data shared by the following sentinel sites	Sector
1.	NVL, MoNFS&R	Public
2.	NRLPD, ASI, NARC	Public
3.	Poultry Research Institute, Rawalpindi, Punjab	Public
4.	Provincial Disease Diagnostic Laboratory, Lahore, Punjab	Public
5.	Disease Investigation Laboratory, Peshawar, KPK	Public
6.	Sindh Institute of Animal Health, Karachi, Sindh	Public
7.	Civil Veterinary Diagnostic Laboratory, Tandojam, Sindh	Public
8.	Disease Investigation Laboratory, Quetta	Public
9.	Central Diagnostic and Analytical Laboratories, K&N's Foods processing plant, Raiwind, Lahore	Private
10.	K&Ns Research and Diagnostic Centre, Abbottabad	Private
11.	SB Labs, Rawalpindi	Private
12.	GP Labs, Lahore	Private
13.	Averroes Labs, Islamabad	Private
14.	Mukhtar Labs, Faisalabad	Private

2.2 Frequency of data submission

Sentinel sites submitted AMR surveillance data to the AMR-CU on a quarterly basis between September 2024 and September 2025. The data submission format and reporting parameters (e.g, AST values, metadata) were agreed upon through consultations with the TWG for AMR. Each sentinel site reported the total number of samples received during the reporting timeframe, as well as the subset of samples that underwent bacterial isolation for *E. coli*, *Salmonella* spp. and *S. aureus* and subsequent AST. Some of the sites were unable to process all submitted samples for bacterial isolation due to resources or protocol constraints. Lack of human resources at private facilities prevented data collection for samples with negative results and it was at their discretion to share data of positive samples. The number of samples tested for each bacterial species was also undeclared by the sentinel sites. Furthermore, the AST panels varied between sites and due to financial constraints limited number of isolates were subjected to AST. In addition, incomplete application of the full AST panel across laboratories was observed.

For samples yielding positive bacterial growth, additional information was submitted using a standardised Microsoft Excel template. This dataset included animal (flock or herd) basic demographic information, laboratory methods used for bacterial isolation and confirmatory testing, and the zone diameter measurements generated using the disk diffusion AST technique. Upon receipt, the AMR-CU conducted extensive data cleaning, compilation, and analysis using R-statistical software. The aggregated findings and interpretations were subsequently presented and reviewed during the TWG's quarterly meetings. The findings were also communicated via News Bulletins.

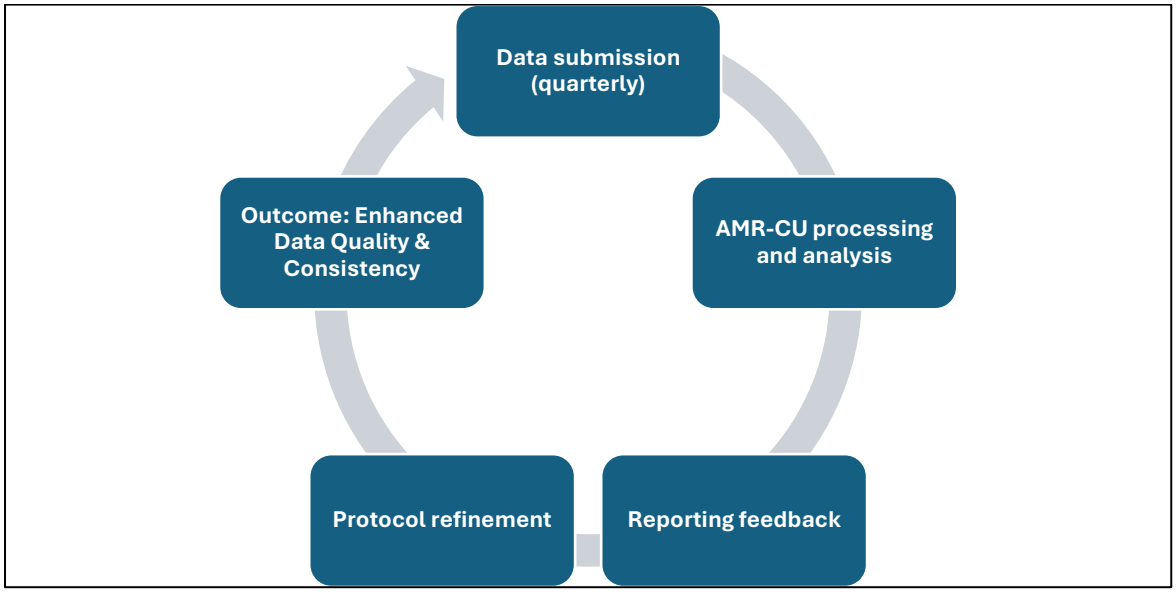

Figure 4: Feedback cycle: Quarterly data submitted by the sentinel sites was processed and analysed by AMR-CU. Issues identified in the data were conveyed to focal persons at the sentinel sites directly as well as presented at TWG forum. The mechanism facilitated in the refinement of data sharing protocols. Ultimately, the feedback facilitated in enhancing data quality and consistency.

A structured feedback mechanism was implemented whereby the focal persons from each laboratory received an assessment of their data quality following each quarterly submission (Figure 4). Over the twelve-month period, four complete rounds of data collection were executed. Concurrently, the format was streamlined and a formalised protocol for data sharing was developed by the AHC office. This iterative process of feedback and procedural standardisation led to a measurable enhancement in the quality and consistency of data submitted to the AMR-CU.

2.3 Target bacteria and animals

Passive surveillance was implemented for three priority microorganisms specified in the 'National Surveillance Strategy for Diseased Food Animals': *Escherichia coli, Salmonella* spp. (non-typhoidal), and *Staphylococcus aureus*. The selection of these microorganisms was determined through a consensus process involving international consultants from AMROH, national consultants, and members of the TWG. Surveillance activities included samples collected from large ruminants (LR) and small ruminants (SR) and poultry (broiler, layer and their breeders. Large ruminant samples were obtained from diseased cattle and buffalo, while SR samples were obtained from goat and sheep.

2.4 Microbiological isolation

2.4.1 Isolation and Identification of *E. coli*

For the isolation of *E. coli,* the samples were first enriched in buffered peptone water (Oxoid, Hampshire, UK) and incubated for 24 hours at 37°C to allow recovery and proliferation of stressed or low-level bacterial cells. Following enrichment, aliquots were streaked onto MacConkey agar (Oxoid, Hampshire, UK) and incubated at 37°C. A single well-isolated colony from each plate, exhibiting typical *E. coli* morphology (smooth, circular, pink lactose-fermenting colonies), was selected as a presumptive *E. coli* isolate. The selected colonies were further sub-cultured on fresh MacConkey agar to ensure purity.

Pure cultures were subjected to conventional biochemical testing. The diagnostic panel included indole, methyl red, Voges–Proskauer, citrate utilization, and triple sugar iron (TSI) tests. All culture media and reagents used for biochemical identification were commercially obtained from Oxoid (Hampshire, UK). Isolates demonstrating indole positivity, methyl red positivity, and typical TSI reactions (acid/acid with gas production), along with negative results for Voges–Proskauer and citrate utilization, were considered as *E. coli*. Further typing/characterisation of the *E. coli* isolates was not conducted, for example, the determination of virulence factors to differentiate the isolates recovered from commensal *E. coli*.

Figure 5: Image of purified E. coli culture on MacConkey agar recovered from poultry faecal sample. These colonies are smooth, circular, pink, lactose-fermenting in nature - a typical characteristic of E. coli on MacConkey agar.

2.4.2 Isolation and Identification of *Salmonella* spp.

For the isolation of *Salmonella* spp., samples were first subjected to pre-enrichment in buffered peptone water (BPW) (Oxoid, Hampshire, UK) and incubated at 37 °C for 24 hours. Following incubation, 0.1 mL aliquots of the BPW culture were transferred into 9 mL of Müller–Kauffmann tetrathionate broth supplemented with brilliant green and novobiocin (MKTTn) (Liofilchem, Italy) and incubated at 42 °C for 24 hours. After selective enrichment, cultures were streaked onto Xylose Lysine Deoxycholate (XLD) agar (Liofilchem, Italy) and Brilliant Green Agar (BGA) (Liofilchem, Italy) plates and incubated at 37 °C for 24 hours.

Presumptive *Salmonella* colonies were identified as black-centered colonies on XLD agar and pink to red colonies surrounded by a red zone on BGA plates[8]. Suspected colonies were picked and purified

[8] Sohail, M. N., Rathnamma, D., Priya, S. C., Isloor, S., Naryanaswamy, H. D., Ruban, S. W., & Veeregowda, B. M. (2021). Salmonella from farm to table: isolation, characterization, and antimicrobial resistance of Salmonella from commercial broiler supply chain and its environment. BioMed Research International, 2021(1), 3987111.

by subculturing on XLD and BGA plates. The purified isolates were subjected to biochemical characterization using Indole, Methyl Red, Triple Sugar Iron (TSI), Voges–Proskauer, citrate utilization, and urease tests. *Salmonella* isolates were typically indole negative and Voges–Proskauer negative, while methyl red and citrate utilization tests were positive. On Triple Sugar Iron (TSI) agar, isolates produced an alkaline slant and acidic butt with hydrogen sulfide (H_2S) production and, in some cases, gas formation. All the isolates were urease negative. These biochemical profiles were considered confirmatory for *Salmonella* spp. in accordance with established diagnostic criteria[9,10]. Serogrouping or serotyping of the *Salmonella* isolates was not done. All isolates were deemed as non-typhoidal salmonellae.

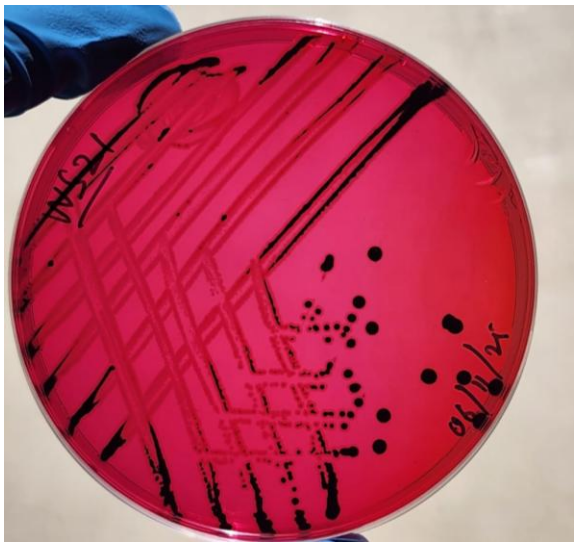

Figure 6: Image of purified Salmonella culture on Xylose Lysine Deoxycholate (XLD) agar recovered from poultry faecal sample. These colonies are black centred with pinkish culture media, a typical characteristic of Salmonella on XLD agar.

2.4.3 Isolation and Identification of *Staphylococcus aureus*

For the isolation of *Staphylococcus aureus,* the samples were enriched in nutrient broth (Oxoid, Hampshire, UK) and incubated for 24 hours at 37°C. Following enrichment, aliquots were streaked onto Mannitol Salt Agar (Oxoid, Hampshire, UK) and incubated at 37°C. On MSA, presumptive *S. aureus* colonies appeared as smooth, round, convex, golden-yellow colonies resulting from mannitol fermentation[11].

Suspected colonies were picked and purified on fresh MSA plates and subjected to microscopic and biochemical characterization. Gram staining revealed Gram-positive cocci arranged in grape-like clusters. Biochemical identification showed positive catalase and coagulase reactions, while isolates were oxidase negative. These colony characteristics and biochemical profiles were considered confirmatory for *Staphylococcus aureus* following standard microbiological identification criteria[12].

[9] Siddique, A., Azim, S., Ali, A., Andleeb, S., Ahsan, A., Imran, M., & Rahman, A. (2021). Antimicrobial resistance profiling of biofilm forming non typhoidal Salmonella enterica isolates from poultry and its associated food products from Pakistan. Antibiotics, 10(7), 785.

[10] Rahman, M. A., Ahmad, T., Mahmud, S., Barman, N. C., Haque, M. S., Uddin, M. E., & Ahmed, R. (2019). Isolation, identification and antibiotic sensitivity pattern of Salmonella spp. from locally isolated egg samples. Am. J. Pure Appl. Sci, 1(1), 1-11.

[11] Bude, S. A., & Mengesha, A. K. (2021). Isolation and identification of Staphylococcus aureus from dairy farms in Bishoftu Town, Ethiopia.

[12] Shahzad, M. A., Yousaf, A., Ahsan, A., Irshad, H., Riaz, A., Khan, A., ... & Javed, S. (2024). Virulence and resistance profiling of Staphylococcus aureus isolated from subclinical bovine mastitis in the Pakistani Pothohar region. Scientific Reports, 14(1), 14569.

Figure 7: Image of purified Staphylococcus aureus culture on Mannitol Salt Agar (MSA) recovered from cattle milk sample. These colonies are typically smooth, round and golden-yellow on MSA.

2.5 Antimicrobial Susceptibility Testing

The positive isolates were subjected to AST using the Kirby-Bauer disc diffusion test following the recommendations of the Clinical Laboratory Standard Institute (CLSI)[13]. Briefly, 2-3 well-isolated colonies from the identified pure isolates were emulsified into 5 mL of sterile normal saline solution, and the turbidity of the solution was adjusted to that of a 0.5 McFarland standard. A sterile cotton swab was immersed in normal saline suspension and uniformly streaked onto a Mueller-Hinton agar medium (Oxoid, UK) plate. Following this, antibiotic discs (Oxoid, UK) containing a specific concentration of antibiotics were placed on the agar surface, and the plates were incubated at 37°C for 24 hours. Interpretation of mm inhibition zone values was in accordance with CLSI guidelines using VET 01S (for pathogens isolated from diseased terrestrial animals)[14] or CLSI M100 (35th Edition)[13], where applicable and where interpretative criteria were available for the antimicrobials included in the AST panel (Annex I - Table 14, Table 15, Table 16).

Fourteen sentinel sites across Pakistan were enrolled in the study. A significant challenge to data harmonisation arose from substantial variation in the AST panels employed at each site, with minimal overlap observed between them. Consequently, it was not feasible to compile AST results against a standardised list of antimicrobials. A further complication at the project's inception was that multiple sites recorded only qualitative interpretive categories—Resistant (R), Susceptible (S), or Intermediate (I)—rather than quantitative zone diameters in millimetres (mm). As a result, the data from the initial two quarters from majority of the sites were available solely in this R/I/S format. For the purpose of the current analysis, this qualitative information was retained.

[13] Clinical and Laboratory Standards Institute. CLSI M100: performance standards for antimicrobial susceptibility testing. 35th Ed. (2025).
[14] Clinical and Laboratory Standards Institute. CLSI Vet01S: performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 7th Ed. (2024).

2.6 Statistical Analysis

For each group of animals (LR, SR and poultry), the isolation percentage was calculated separately for *E. coli*, *Salmonella* spp. and *Staphylococcus aureus*.

The AST data was interpreted using CLSI clinical breakpoints, where available for the antimicrobial/s in the AST panel, and classified isolates as resistant (R), intermediate (I) and susceptible (S). A descriptive analysis was then conducted to characterise the AMR phenotypes in LR, SR and poultry. For the calculation of non-susceptible isolates percentage in this surveillance program, isolates exhibiting intermediate susceptibility were grouped with resistant isolates. This aligns with the standard methodology for AMR surveillance, which reports the percentage of non-susceptible isolates to provide a conservative and public health-relevant estimate. Therefore, RIS results were transformed into binary outcome measures (0-susceptible and 1-non-susceptible (resistant/intermediate)). The resulting non-susceptible profiles were contextualised with reference to the WHO priority list of medically important antimicrobials (MIA) - Critically Important Antibiotics (CIA), Highly Important Antibiotics (HIA), Highest Priority Critically Important Antimicrobial (HPCIA)[2].

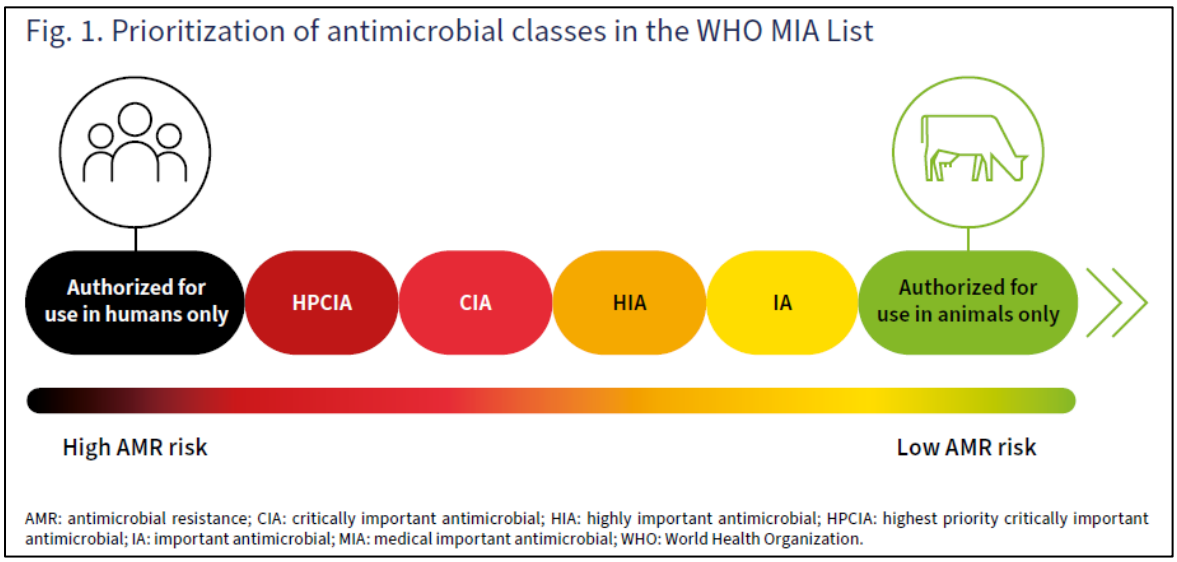

Figure 8: Adapted from WHO's List of Medically Important Antimicrobials: a risk management tool for mitigating antimicrobial resistance due to non-human use. Geneva: World Health Organization; 2024. Licence: CC BY-NC-SA 3.0 IGO. Figure 1. Pg#04[2].

To visualise these profiles, heat maps incorporating hierarchical clustering were generated, employing resistance categories defined by the European Food Safety Authority in the European Union Summary Report. The resistant categories range from 'rare' (<0.1%), 'very low' (0.1%–1.0%), 'low' (>1%–10.0%), 'moderate' (>10.0%–20.0%), 'high' (>20.0%–50.0%), 'very high' (>50.0%–70.0%), to 'extremely high' (>70.0%)[3].

For each bacterial species, multi-class resistance profiles were determined, including those classified as MDR. These profiles were defined as resistance to antimicrobials belonging to at least three or more distinct antimicrobial classes. A summary table was subsequently constructed to detail the specific combinations of antibiotic classes demonstrating these MDR phenotypes.

For all calculated proportions, 95% confidence intervals were derived using the Wilson binomial proportion method, selected for its robust coverage properties with limited sample sizes.

All data was compiled, cleaned and analysed in Microsoft Excel software (Microsoft 365) while the analyses was concurrently performed using the R programming language, version 4.5.1. For visualisation of data R V.4.5.1 was used.

3 Results in diseased FPAs (livestock and poultry)

Passive AMR surveillance was conducted in diseased food-producing animals between September 2024 to September 2025. During the twelve-month surveillance period, the established network of sentinel sites collected a total of 11,915 diagnostic samples for bacteriological analysis. Samples were stratified by production animal group, with a marked predominance of poultry, accounting for 9,494 samples (primarily from broiler, layers and their breeder flocks), compared with 2,421 samples obtained from livestock.

Table 2: Total sample collected through the passive AMR surveillance in livestock and poultry, 2024-2025. A total of 11,915 samples were submitted to the sentinel sites for disease diagnosis. Of these 2,421 samples were from livestock and 9,494 were from poultry.

Animal Group	**Total samples (N)**
Livestock	2,421
a) Large ruminants (LR)	1,605
b) Small ruminants (SR)	816
Poultry	9,494
Total samples	**11,915**

To support a more detailed epidemiological assessment, the livestock category was further stratified by ruminant type, yielding 1,605 samples from LR (cattle and buffalo) and 816 samples from SR (sheep and goats). Given the distinct husbandry practices, pathogen exposure pathways, and AMU patterns across animal groups, a disaggregated analytical approach was applied. Accordingly, the descriptive analysis by sample origin, bacterial isolation percentages, and AMR measurements are presented and discussed separately for poultry, LR, and SR in the subsequent sections.

3.1 Livestock

3.1.1 Bacterial recovery percentages in livestock

Figure 9 presents the recovery of *E. coli*, *Salmonella* spp., and *S. aureus* from diseased LR and SR.

From the 1,605 LR samples received, 203 *E. coli* isolates were recovered corresponding to a minimum prevalence of 12.6% (95% CI: 11.1 – 14.3%). In addition, *Salmonella* spp. were isolated from 51 samples (3.2%, 95% CI: 2.4 - 4.1%) and *S. aureus* was detected in 195 samples (12.1%, 95% CI: 10.6 - 13.9%).

Analysis of the 816 SR samples yielded 56 *E. coli* isolates (6.9%, 95% CI: 5.3 - 8.8%), 26 *Salmonella* spp. isolates (3.2%, 95% CI: 2.2 - 4.6%), and 42 *S. aureus* isolates (5.1%, 95% CI: 3.8 - 6.8%).

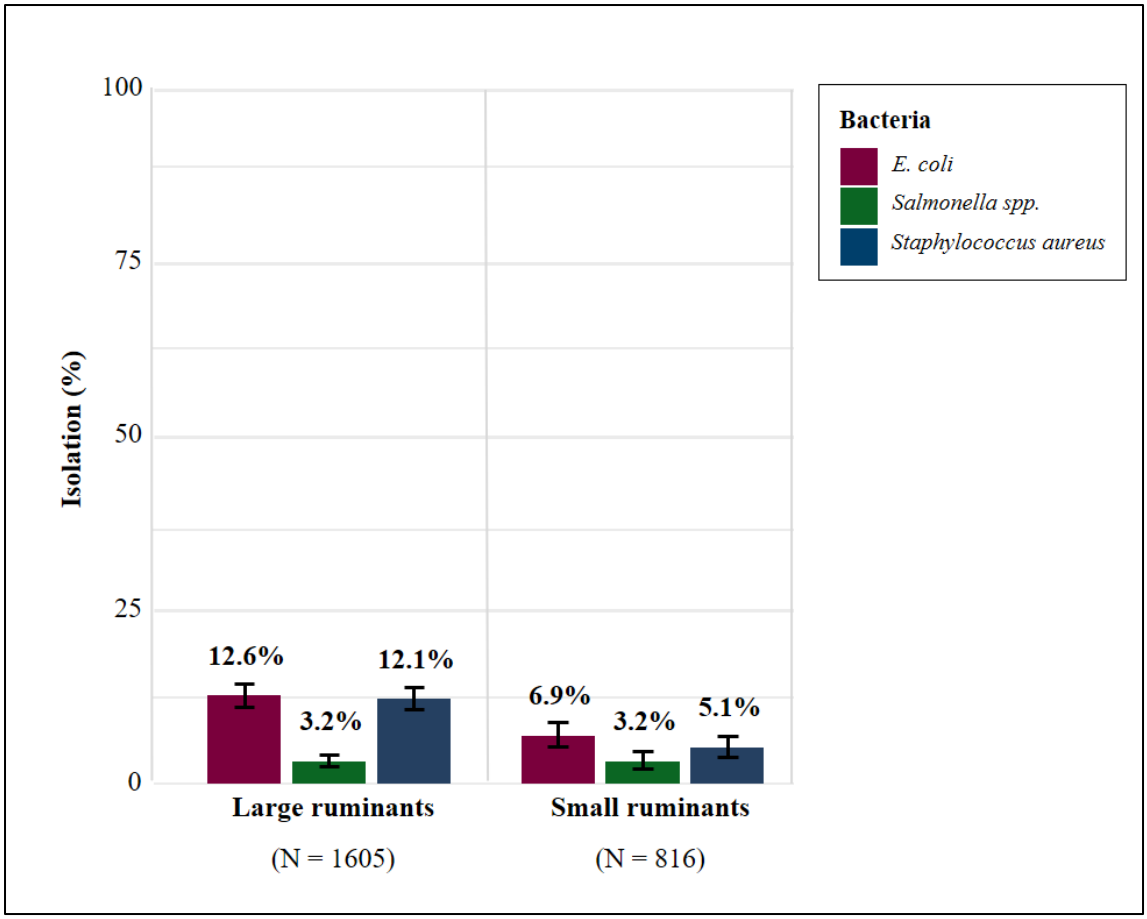

Figure 9: Recovery of bacteria from large and small ruminant samples, percentages and 95% confidence intervals.

A comparative analysis of the isolation percentages between ruminant groups revealed a distinct pattern. The prevalence of both *E. coli* and *S. aureus* though highly underestimated because of the study limitations, was approximately twice as high in LR samples as in SR samples (Table 3).

Table 3: The minimum prevalence of E. coli, Salmonella spp. and S. aureus in small and large ruminants with 95% confidence interval.

Bacteria	Animal Group	Total samples (N)	Isolate (n)	Minimum prevalence [(n/N)*100]	95% Confidence interval
E. coli	Large ruminants	1605	203	12.6	11.05 - 14.33
Salmonella spp.		1605	51	3.2	2.42 - 4.13
Staphylococcus aureus		1605	195	12.1	10.58 - 13.87
E. coli	Small ruminants	816	56	6.9	5.32 - 8.8
Salmonella spp.		816	26	3.2	2.16 - 4.59
Staphylococcus aureus		816	42	5.1	3.77 - 6.79

Only a subset of the positive isolates underwent AST because of resources or protocol constraints of the individual laboratories. The following flow charts present a clear breakdown (Figure 10, Figure 11).

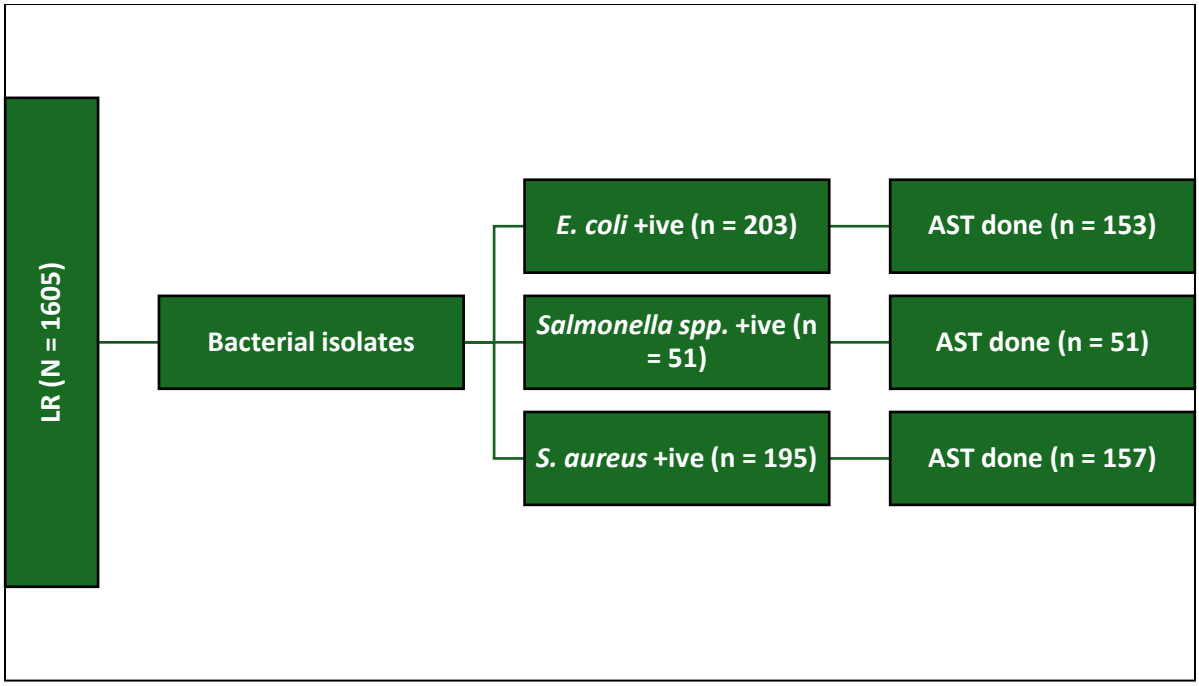

Figure 10: Flow chart shows the data for Large ruminants (LR). Not all samples submitted to sentinel sites underwent bacterial isolation, and neither did all bacterial isolates underwent AST.

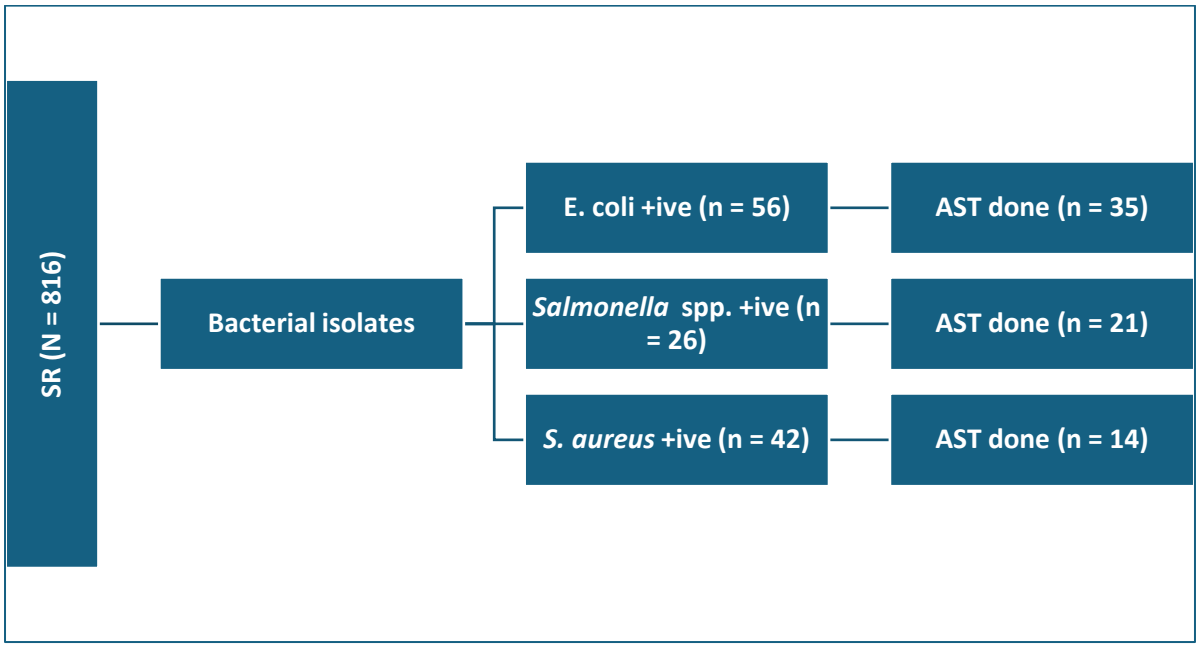

Figure 11: Flow chart shows the data for small ruminants (SR). Not all samples submitted to sentinel sites underwent bacterial isolation, and neither did all bacterial isolates underwent AST.

3.1.2 Bacterial sample types

Among *E. coli* isolates recovered from LR, the majority originated from milk samples, accounting for 81.7% of all samples. Other sources, including faeces (4.6%) and blood (3.3%), contributed only marginally. This distribution indicates a strong association between *E. coli* isolation and mastitis, with a smaller proportion of cases likely reflecting systemic infection in

dairy herds. This finding underscores the importance of *E. coli* in the pathogenesis of mastitis and its relevance in AMR surveillance in bovine animals, specifically in dairy systems.

Salmonella spp. isolates presented a more diverse origin. Milk remained a significant source (25.5%), alongside a notable proportion from "Other" categories (21.6%). Important sample matrices included liver and faeces (both 9.8%), as well as heart, kidney, and lung (each 5.9%), indicative of potential systemic (i.e., from organs) and enteric (i.e., from faeces) nature of the *Salmonella* spp. recovered. Capacity for serotyping should be prioritised to determine the common serotypes of ruminants and their zoonotic significance.

The recovery of *S. aureus* was revealed homogeneity and highly concentrated in milk samples, which accounted for 91.7% of all isolates. All other sample types, including lung, liver, and "Other" categories, each represented 1.9% or less. This distribution underscores the dominant role of *S. aureus* as a principal etiologic agent of bovine mastitis. (Annexe II, Table 18)

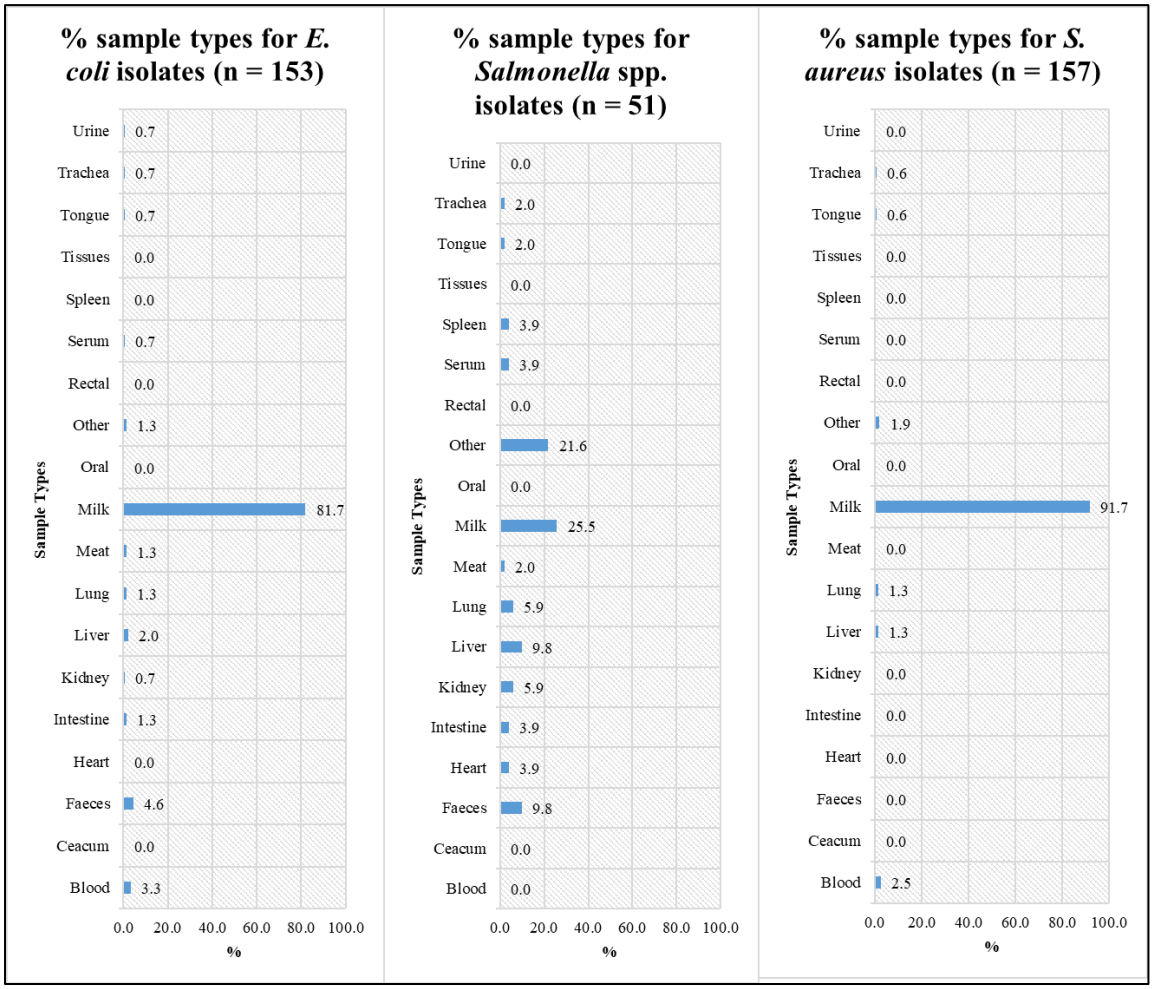

Figure 12: The distribution of sample types for LR bacterial isolates. Milk was the predominant sample type for E. coli and S. aureus underscroing their importance in passive AMR surveillance in ruminants.

In SR, *E. coli* isolates displayed a more heterogeneous distribution across specimen types, with the highest proportion recovered from lung samples (20.0%). Substantial recovery was also observed from kidney, liver, spleen (each 14.3%), and faeces (14.3%). Milk was a minor source at 5.7%, indicating diverse infection or carriage sites.

Salmonella spp. isolation was most frequent from the lungs (28.6%), followed by the liver (23.8%) and kidney (19.0%). A notable proportion also originated from the spleen (14.3%). This pattern suggests a primary systemic and respiratory involvement in these hosts.

For *S. aureus*, the primary reservoirs were milk (42.9%) and oral samples (35.7%). Other sites, including liver, lung, and spleen, each accounted for 7.1% of isolates. This indicates a significant association with mastitis and oral carriage within sheep and goat populations. (Annexe II, Table 19)

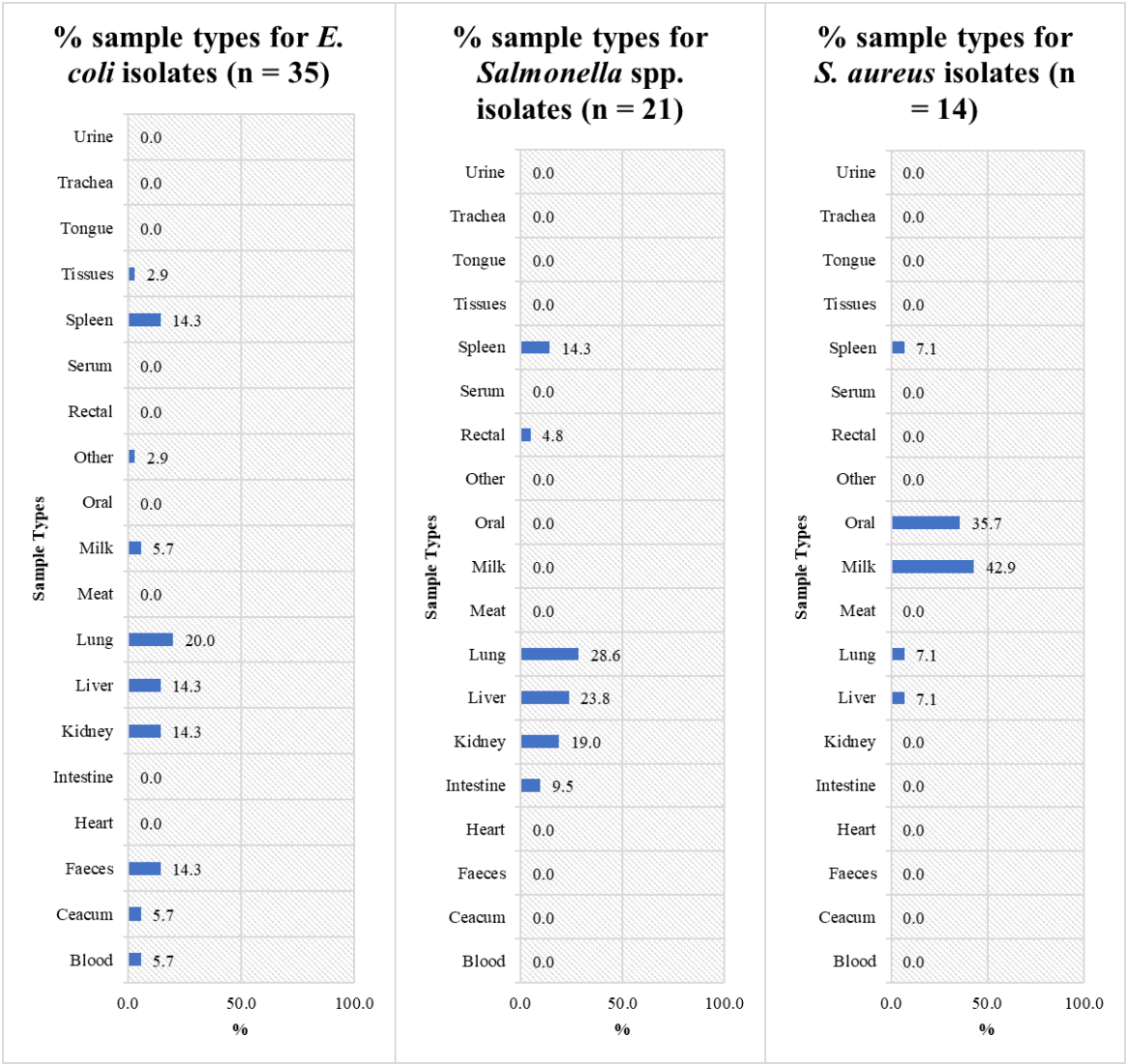

Figure 13: The distribution of sample type for SR bacterial isolates, indicating a more heterogeneous sample matrices.

3.1.3 AMR phenotypes in livestock

3.1.3.1 Heat map analysis of AST patterns of bacterial isolates in livestock

The AST phenotypes of isolates for *E. coli*, *Salmonella* spp., and *S. aureus* were analysed. To synthesise and interpret the complex resistance profiles, the numerical resistance percentages for each pathogen-antibiotic combination were visualised using a hierarchical clustering heat map. This analytical tool employed the categorisation framework of the EUSR, translating the quantitative data into an intuitive colour gradient. The gradient corresponds

to predefined resistance categories, ranging from '**Rare**' (dark blue) to '**Extremely High**' (red). Hierarchical clustering was applied independently to both dimensions of the matrix, organising the antibiotics (rows) and the bacterial species (columns) based on the similarity of their resistance profiles. Furthermore, the antibiotics were annotated according to the WHO's categorisation of medically important antimicrobials (MIA). The resultant visualisation reveals distinct clustering patterns, revealing resistance profiles across the studied pathogens.

3.1.3.2 E. coli isolates in livestock

The heat map presented in Figure 14 illustrates the AMR profile of *E. coli* isolates from LR and SR against thirteen and twelve antibiotics, respectively. The overall resistance patterns observed for LR and SR were notably convergent, with the majority of antibiotics displaying **high** to **extremely high** levels of resistance. It is imperative to note that, due to a lack of harmonisation in antimicrobial susceptibility testing (AST) panels across sentinel sites, coupled with the inconsistent application of these panels to all isolates, the number of isolates tested for certain antibiotics (see the value of 'n' within each coloured cell of Figure 14) was extremely low. This paucity of data has the effect of inflating the calculated resistance percentages, which must therefore be interpreted with considerable caution. It is strongly advised that any conclusions drawn from these particular figures are tempered by an awareness of this significant methodological limitation. This applies to the entire document.

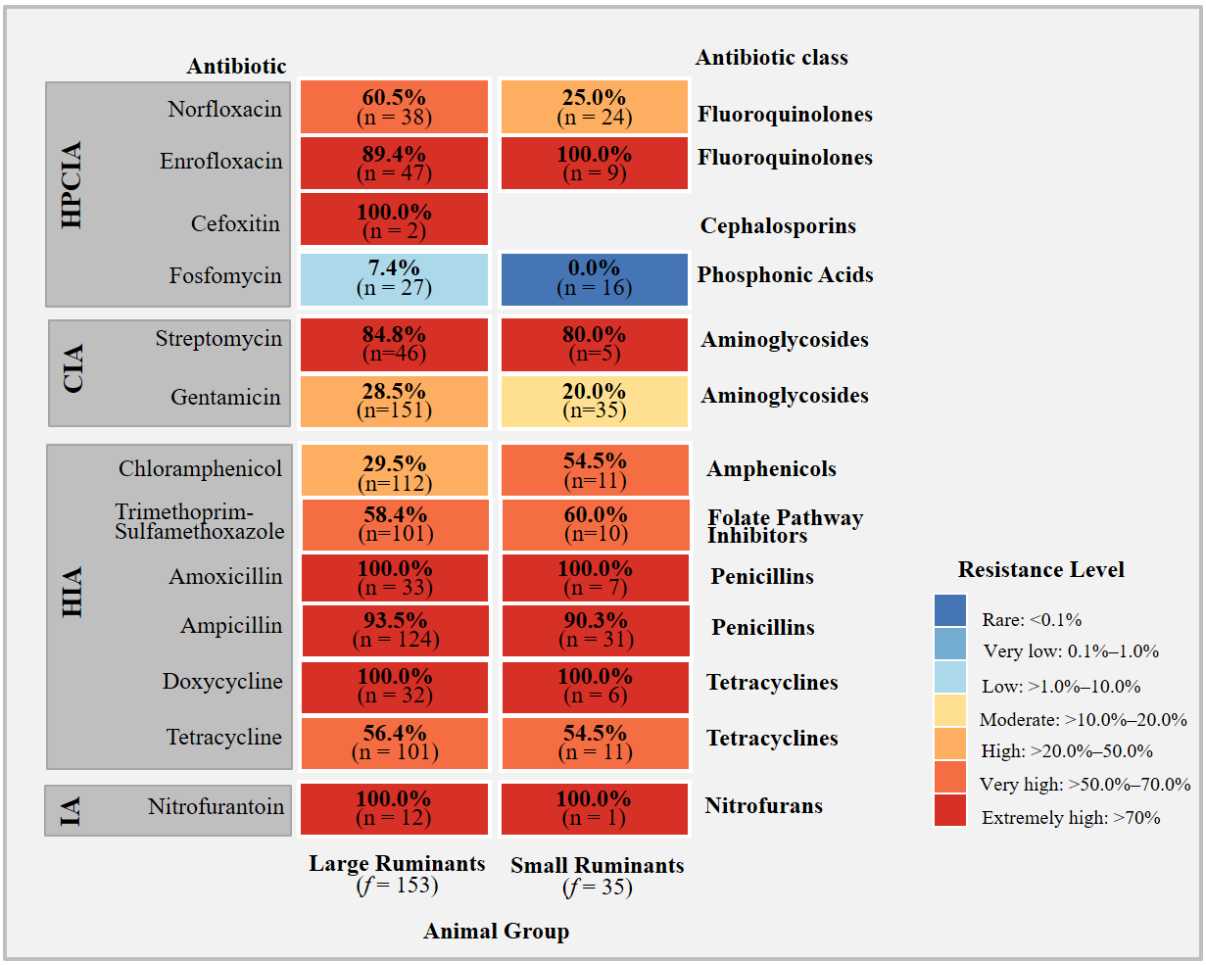

Figure 14: Heat map of resistance percentages in E. coli isolated in LR and SR. The legend uses the EUSR's categorisation system. The antibiotics are classified based on WHO's MIA categories. 'n' in each cell refers to total count of isolates tested against each antibiotic demonstrating the need for harmonization of AST panels. 'f' refers to total isolates.

For LR isolates, twelve of the thirteen tested antibiotics—specifically norfloxacin, enrofloxacin, cefoxitin, streptomycin, gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole, amoxicillin, ampicillin, doxycycline, tetracycline, and nitrofurantoin—exhibited resistance categorised as **moderate** to **extremely high**. A highly similar profile was observed for SR, where eleven of the twelve antibiotics (the same panel excluding cefoxitin) fell within this elevated resistance range.

Analysis focused on the WHO categories of Highest Priority Critically Important Antimicrobials (HPCIAs) and Critically Important Antimicrobials (CIAs) revealed pronounced resistance:

1. **HPCIAs:** Against norfloxacin, resistance was **very high** in LR (n = 38) and **high** in SR (n = 24). Resistance to enrofloxacin was **extremely high** in both host groups (LR: n = 47; SR: n = 9). Cefoxitin, tested only in LR, also showed **extremely high** resistance.

2. **CIAs:** Streptomycin demonstrated **extremely high** resistance in both LR (n = 46) and SR (n = 5). Resistance to gentamicin was **high** in LR (n = 151) and moderate in SR (n = 35).

It is important to note that the particularly high resistance estimates for some SR categories were based on a limited number of isolates. Consequently, these specific values may be susceptible to overestimation and must be interpreted with caution.

3.1.3.3 *Salmonella spp. isolates in livestock*

The heat map presented in Figure 15 illustrates the AMR profile of *Salmonella* spp. isolates from LR and SR to eight antibiotics belonging to 6 classes. The overall resistance patterns observed for LR and SR were again notably similar, with the majority of antibiotics displaying **high** to **extremely high** levels of resistance.

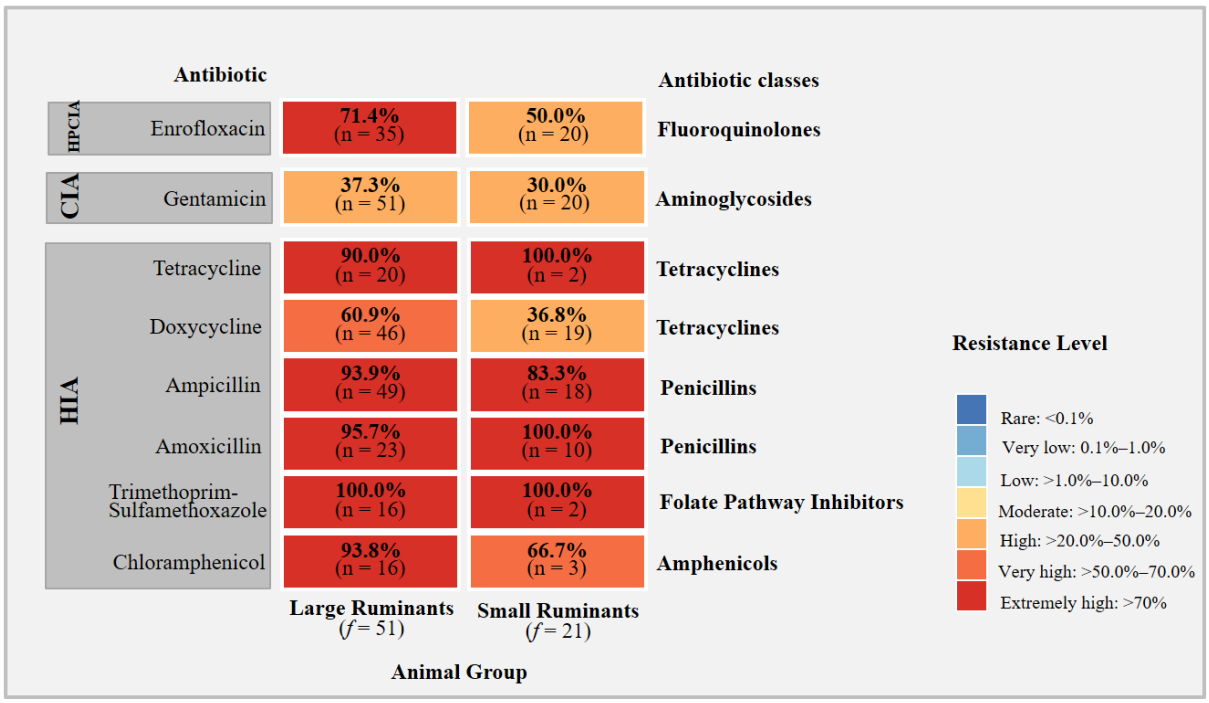

Figure 15: Heat map of resistance percentages in Salmonella spp. isolated in LR and SR. The legend uses the EUSR's categorisation system. The antibiotics are classified based on WHO's MIA categories. 'n' in each cell refers to total count of isolates tested against each antibiotic demonstrating the need for harmonization of AST panels. 'f' refers to total isolates.

For LR isolates, all eight antibiotics—specifically enrofloxacin, gentamicin, tetracycline, doxycycline, ampicillin, amoxicillin, trimethoprim-sulfamethoxazole, and chloramphenicol—exhibited resistance categorised as **high** to **extremely high**. An exceedingly similar profile was observed for SR, where all eight antibiotics fell within this elevated resistance range.

Analysis focused on the WHO categories of HPCIAs and CIAs revealed pronounced resistance:

1. **HPCIAs:** Resistance to enrofloxacin was **extremely high** in LR (n = 35) and very high in SR (n = 20)
2. **CIAs:** Resistance to gentamicin was **high** in both groups (LR: n = 51; SR: n = 20).

Similar to *E. coli*'s resistance patterns, particularly high resistance percentages calculated for some LR and SR categories are based on a limited number of isolates.

3.1.3.4 *Staphylococcus aureus isolates in livestock*

The heat map presented in Figure 16 illustrates the AMR profile of *S. aureus*. isolates from LR and SR to eleven antibiotics belonging to 8 classes. The overall resistance patterns observed for LR and SR were yet again comparable to both *E. coli* and *Salmonella* spp., with the majority of antibiotics displaying **moderate** to **very high** levels of resistance.

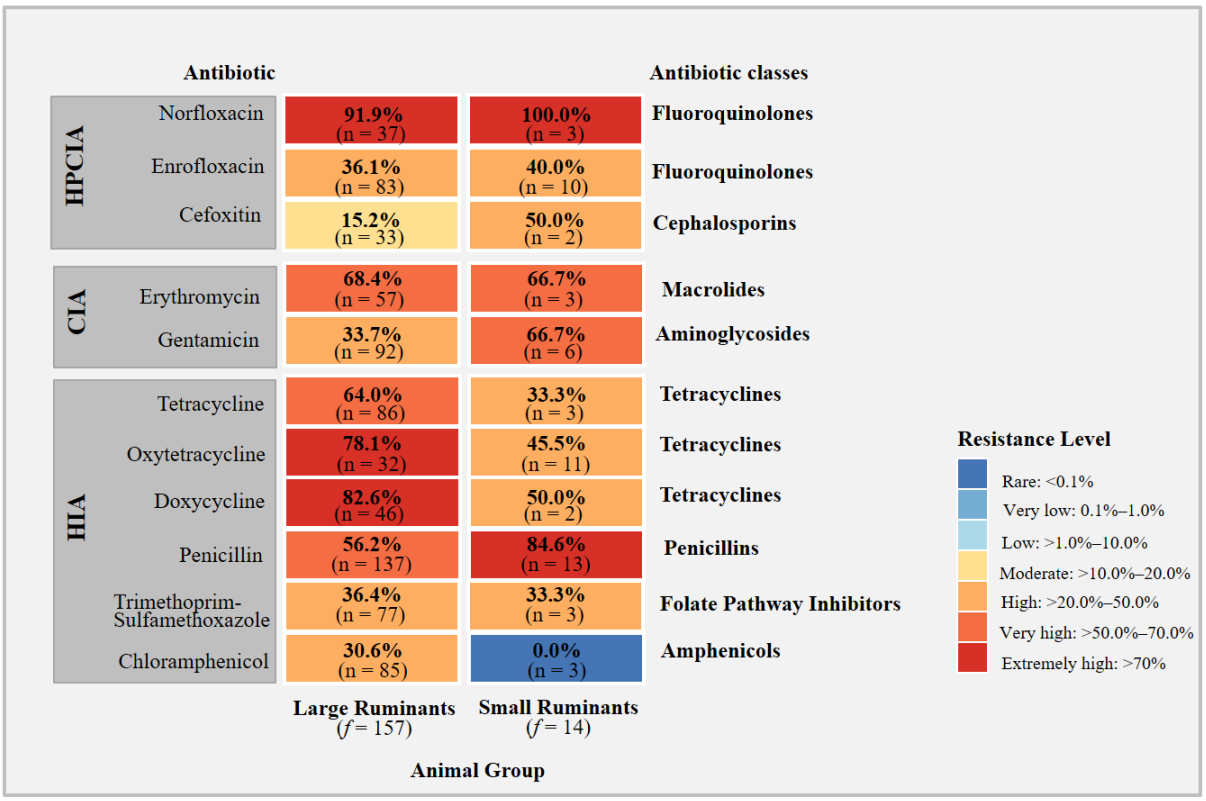

Figure 16: Heat map of resistance percentages in S. aureus isolated in LR and SR. The legend uses the EUSR's categorisation system. The antibiotics are classified based on WHO's MIA categories. 'n' in each cell refers to total count of isolates tested against each antibiotic demonstrating the need for harmonization of AST panels. 'f' refers to total isolates.

For LR isolates, all eleven antibiotics—specifically norfloxacin, enrofloxacin, cefoxitin, erythromycin, gentamicin, tetracycline, oxytetracycline, doxycycline, penicillin, trimethoprim-sulfamethoxazole, and chloramphenicol—exhibited resistance categorised as **moderate** to **extremely high**. A decidedly comparable profile was observed for SR, where all eight antibiotics (with the exception of chloramphenicol) fell within this elevated resistance range.

Analysis focused on the WHO categories of HPCIAs and CIAs revealed pronounced resistance:

1. **HPCIAs:** Resistance to norfloxacin was **extremely high** in both groups (LR: n = 37; SR: n = 3). Resistance to enrofloxacin was also **high** in both group (LR: n = 83; SR: n = 10). Lastly resistance was **moderate** to cefoxitin in LR (n = 33) and **very high** in SR (n = 2)
2. **CIAs:** Resistance to erythromycin was **very high** in both groups (LR: n = 57; SR: n = 3), whereas in gentamicin resistance was **high** in LR (n = 92), and **very high** in SR (n = 6)

Similar to previous two bacterial resistance patterns, high resistance percentages calculated for SR categories are based on a limited number of isolates.

3.1.4 AMR patterns based on WHO priority classification of bacterial isolates in livestock

3.1.4.1 E. coli AMR in LR

Figure 17 illustrates the AMR profile of 153 *E. coli* isolates recovered from diseased LR. Antibiotics are categorised according to the WHO classifications of HPCIA, CIA, HIA, and IA. Resistance varied substantially, ranging from 7.4% to 100.0%. The number of bacterial isolates of LR that underwent AST against certain antibiotics was very low (such as cefoxitine and

nitrofurantoin). This leads to very large confidence intervals (Figure 17). The results must be interpreted with caution.

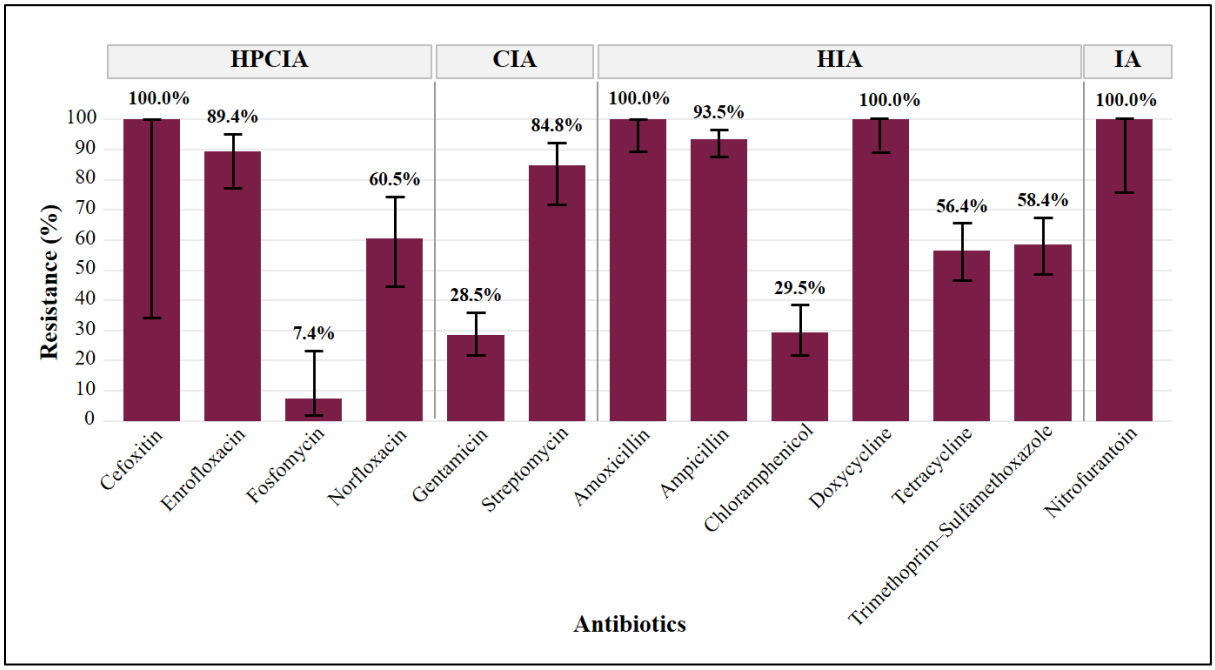

Figure 17: AST pattern of E. coli isolates in LR (n = 153) by antibiotics and WHO's MIA categorisation.

In the HPCIA category, cephalosporin and fluoroquinolones showed variable resistance: cefoxitin 100.0% (95% CI: 34.2 - 100%), norfloxacin 60.5% (95% CI: 44.7 - 74.4%), and enrofloxacin 89.4% (95% CI: 77.4 - 95.4%).

For CIA agents, aminoglycosides showed distinct resistance: gentamicin demonstrated 28.5% resistance (95% CI: 21.9 - 36.1%) and streptomycin 84.8% (95% CI: 71.8 - 92.4 %).

Among HIA compounds, amoxicillin showed absolute resistance at 100% (95% CI: 89.6 - 100%) as well as and doxycycline (95% CI: 89.3 - 100%), with ampicillin at near absolute resistance as well as 93.5% (95% CI: 87.8 - 96.7%). Resistance to other HIA agents was: chloramphenicol 29.5% (95% CI: 21.8 - 38.5%), trimethoprim-sulphamethoxazole 58.4% (95% CI: 48.7 - 67.5%), and tetracycline 56.4% (95% CI: 46.7 - 65.7%).

Of the HI Agents, 100% resistance was observed against Nitrofurantoin (95% CI: 75.8 – 100%) (Annexe II - Table 20)

3.1.4.2 E. coli AMR in SR

Figure 18 illustrates the AMR profile of 35 *E. coli* isolates recovered from diseased SR. Resistance varied substantially, ranging from 0.0% to 100.0%. The number of bacterial isolates of SR that underwent AST was very low. This leads to very large confidence intervals (Figure 18). The results must be interpreted with caution.

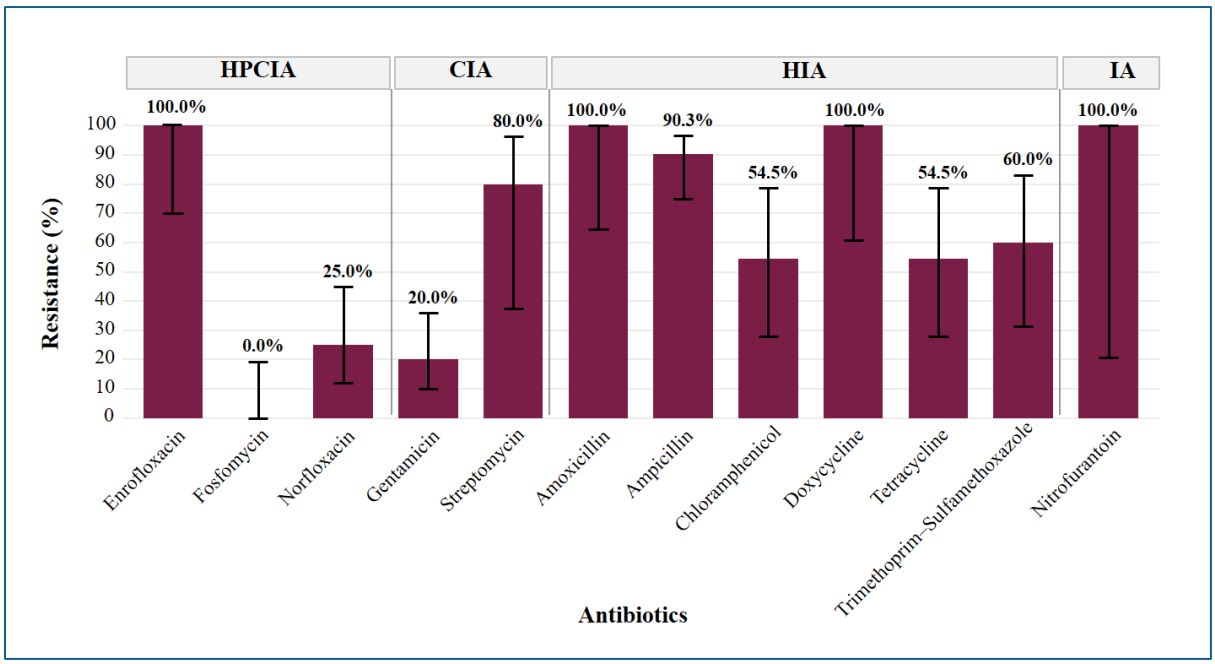

Figure 18: AST pattern of E. coli isolates in SR (n = 35) by antibiotics and WHO's MIA categorisation.

In the HPCIA category, fluoroquinolones showed variable resistance: norfloxacin 25% (95% CI: 12 - 44.9%), and enrofloxacin 100% (95% CI: 70.1 - 100%).

For CIA agents, aminoglycosides showed distinct resistance: gentamicin demonstrated 20% resistance (95% CI: 10 - 35.9%) and streptomycin 80% (95% CI: 37.6 - 96.4%).

Among HIA compounds, similar to LR, amoxicillin showed absolute resistance at 100% (95% CI: 64.6 - 100%) as well as and doxycycline (95% CI: 61 - 100%), with ampicillin at near absolute resistance as well as 90.3% (95% CI: 75.1 - 96.7%). Resistance to other HIA agents was: chloramphenicol 54.5% (95% CI: 28 - 78.7%), trimethoprim-sulfamethoxazole 60% (95% CI: 31.3 - 83.2%), and tetracycline 54.5% (95% CI: 28 - 78.7%).

In the HI agents, 100% resistance was observed against Nitrofurantoin (95% CI: 20.7 - 100%)

The large confidence intervals are a clear indication of very small sample size resistant to the enlisted antibiotics (Annexe II - Table 20).

3.1.4.3 Salmonella spp. AMR in LR

Figure 19 delineates the AMR profile for a panel of 51 *Salmonella* spp. isolates recovered from diseased LR. A substantial variation in resistance prevalence was observed across the tested antimicrobial agents, ranging from 37.3% to 100.0%.

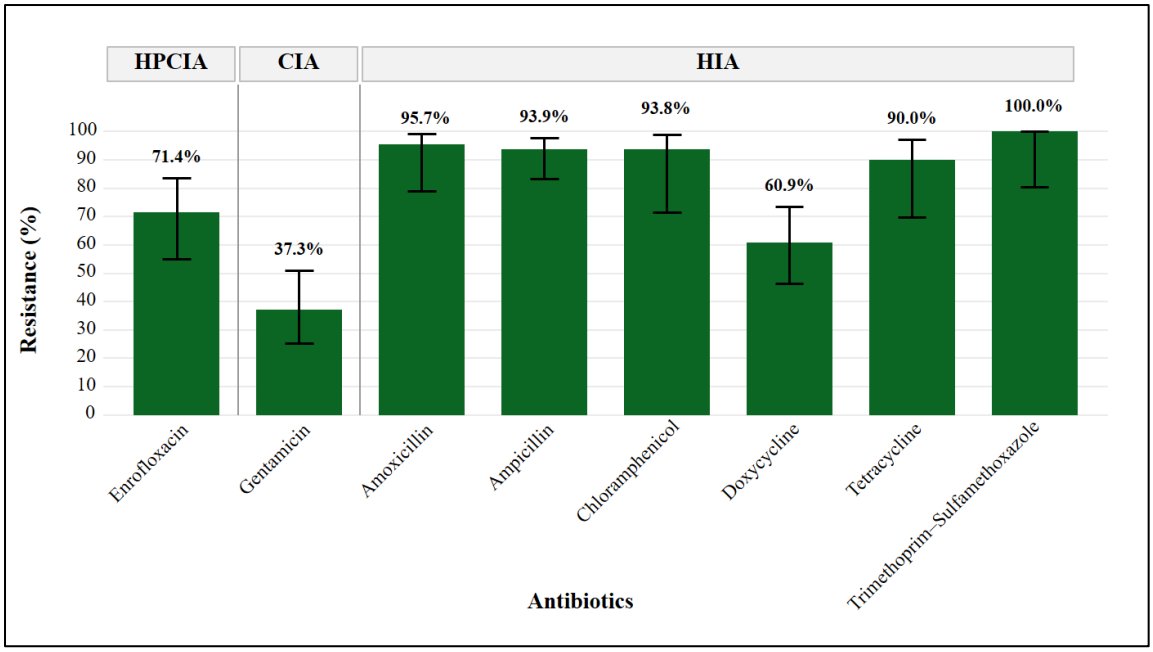

Figure 19: AST pattern of Salmonella spp. isolates in LR (n = 51) by antibiotics and WHO's MIA categorisation.

Concerning agents classified as HPCIAs, resistance to enrofloxacin was recorded at 71.4% (95% CI: 54.9 - 83.7%).

For the CIA agent gentamicin, a resistance prevalence of 37.3% (95% CI: 25.3 - 51.0%) was demonstrated.

Among the HIA compounds, resistance was notably high for several agents: amoxicillin at 95.7% (95% CI: 79.0 - 99.2%), ampicillin at 93.9% (95% CI: 83.5 - 97.9%), and chloramphenicol at 93.8% (95% CI: 71.7 - 98.9%). Elevated resistance was also observed for tetracycline at 90.0% (95% CI: 69.9 - 97.2%). Lower, though still significant, resistance was noted for doxycycline at 60.9% (95% CI: 46.5 - 73.6%). Complete resistance, at 100.0%, was identified for trimethoprim-sulphamethoxazole (95% CI: 80.6 - 100.0%).

No agents categorised as HI were reported as having been tested by the participating laboratories (Annexe II - Table 21).

3.1.4.4 Salmonella spp. AMR in SR

Figure 20 presents the AMR profile of 21 *Salmonella* spp. isolates obtained from diseased SR. Resistance prevalence exhibited considerable variation, spanning from 30.0% to 100.0% across the antimicrobials tested. The number of bacterial isolates of SR that underwent AST was very low. This leads to very large confidence intervals (Figure 20). The results must be interpreted with caution.

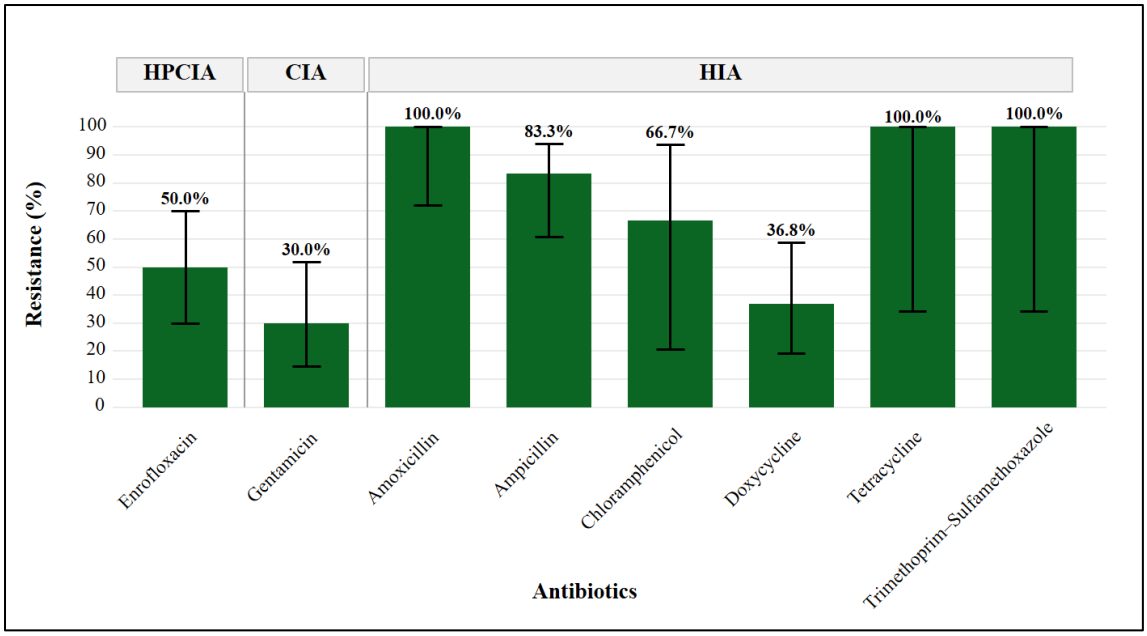

Figure 20: AST pattern of Salmonella spp. isolates in SR (n = 21) by antibiotics and WHO's MIA categorisation.

Within the HPCIA category, resistance to enrofloxacin was 50.0% (95% CI: 29.9 - 70.1%).

For the CIA gentamicin, resistance was demonstrated at 30.0% (95% CI: 14.5 - 51.9%).

Among HIA compounds, complete resistance was identified for trimethoprim-sulfamethoxazole (100.0%; 95% CI: 34.2 - 100.0%), amoxicillin (100.0%; 95% CI: 72.2 - 100.0%) and tetracycline (100.0%; 95% CI: 34.2 - 100.0%). Resistance to ampicillin was also extremely high at 83.3% (95% CI: 60.8 - 94.2%), while chloramphenicol showed resistance at 66.7% (95% CI: 20.8 - 93.9%). Lower, yet notable, resistance was recorded for doxycycline at 36.8% (95% CI: 19.1 - 59.0%).

No HI agents were reported as tested by the participating laboratories (Annexe II - Table 21).

3.1.4.5 Staphylococcus aureus AMR in LR

Figure 21 illustrates the AMR characteristics of 157 *Staphylococcus aureus* isolates derived from clinical LR cases. The prevalence of resistance demonstrated a wide variation, extending from 15.2% to 91.9% among the evaluated antimicrobials.

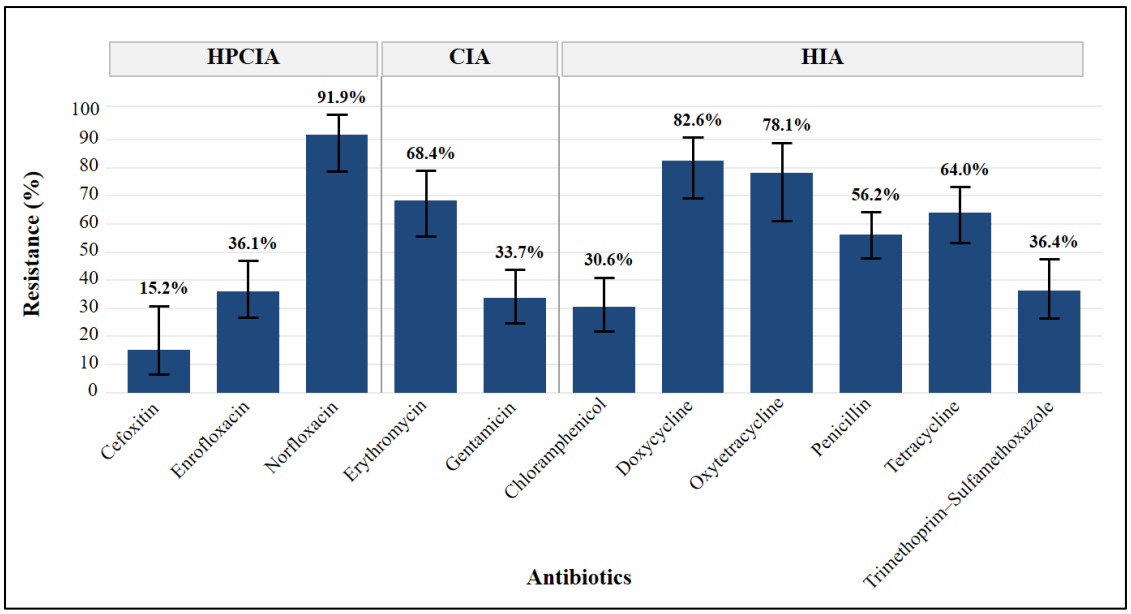

Figure 21: AST pattern of Staphylococcus aureus isolates in LR (n = 157) by antibiotics and WHO's MIA categorisation

In the HPCIA classification, the resistance level for cefoxitin was measured at 15.2% (95% CI: 6.7–30.9%). Conversely, resistance to the fluoroquinolones was markedly greater, with values of 36.1% (95% CI: 26.6–46.9%) for enrofloxacin and 91.9% (95% CI: 78.7–97.2%) for norfloxacin.

For CIA agents, erythromycin resistance stood at 68.4% (95% CI: 55.5–79.0%), whereas gentamicin resistance was lower at 33.7% (95% CI: 24.9–43.8%).

Concerning HIA compounds, the most substantial resistance was evidenced against doxycycline (82.6%; 95% CI: 69.3–90.9%) and oxytetracycline (78.1%; 95% CI: 61.2–89.0%). Resistance to tetracycline was 64.0% (95% CI: 53.4–73.3%), and to penicillin 56.2% (95% CI: 47.8–64.2%). More modest yet significant resistance levels were documented for trimethoprim-sulphamethoxazole (36.4%; 95% CI: 26.5–47.5%) and chloramphenicol (30.6%; 95% CI: 21.8–41.0%).

In keeping with the other profiles, no testing of HI agents was reported by the contributing laboratories (Annexe II - Table 22).

3.1.4.6 Staphylococcus aureus AMR in SR

Figure 22 delineates the AMR profile for a panel of 14 *Staphylococcus aureus* isolates recovered from diseased SR. The data reveal a pronounced disparity in resistance rates, which extend from 0.0% to 100.0%. The number of bacterial isolates of SR that underwent AST was very low. This leads to very large confidence intervals (Figure 21). The results must be interpreted with caution.

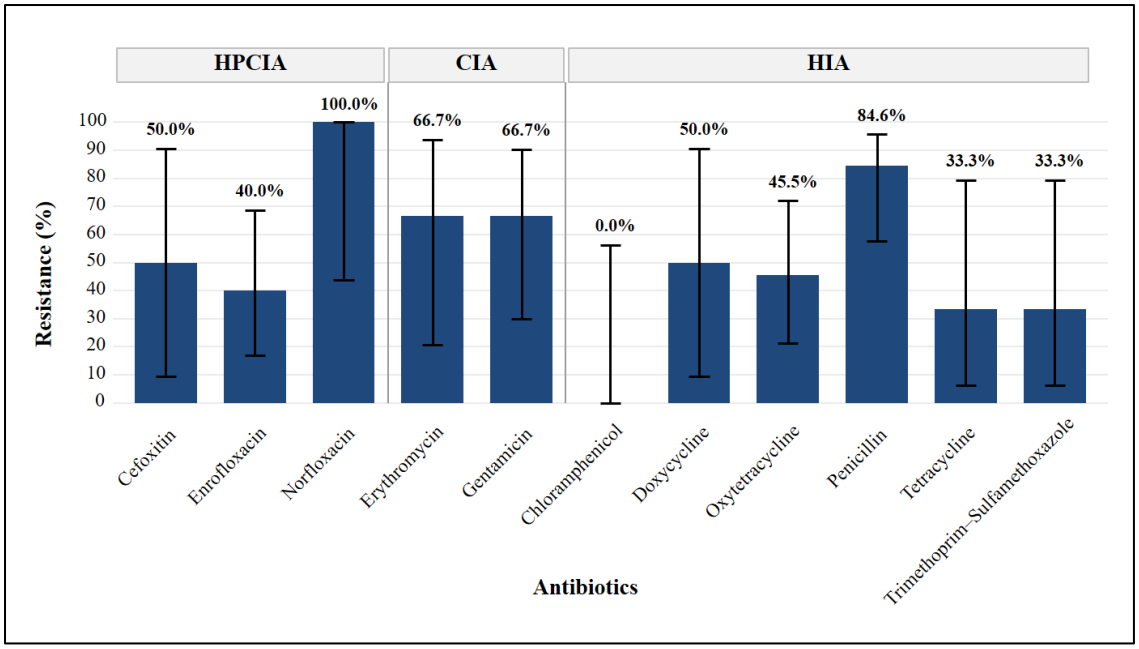

Figure 22: AST pattern of Staphylococcus aureus isolates in SR (n = 14) by antibiotics and WHO's MIA categorisation

Within the HPCIA category, resistance to the cephalosporin, cefoxitin, was 50.0% (95% CI: 9.5 - 90.5%). For fluoroquinolones, resistance to enrofloxacin was 40.0% (95% CI: 16.8 - 68.7%), while complete resistance was observed for norfloxacin at 100.0% (95% CI: 43.9 - 100.0%).

In the CIA category, identical resistance rates of 66.7% were documented for both gentamicin (95% CI: 30.0 - 90.3%) and erythromycin (95% CI: 20.8 - 93.9%).

Among HIA compounds, the highest resistance was evident for penicillin at 84.6% (95% CI: 57.8 - 95.7%). For tetracycline, resistance varied considerably: doxycycline was 50.0% (95% CI: 9.5–90.5%), oxytetracycline 45.5% (95% CI: 21.3 - 72.0%), and tetracycline 33.3% (95% CI: 6.1–79.2%). Resistance to trimethoprim-sulphamethoxazole was also 33.3% (95% CI: 6.1–79.2%). In contrast, no resistance was detected for chloramphenicol (0.0%; 95% CI: 0.0–56.1%) (Annexe II - Table 22).

3.1.5 Multi-class resistance and MDR profile of bacterial isolates in livestock

3.1.5.1 E. coli in livestock

Employing the definition established by Magiorakos et al., (2012), whereby multidrug resistance (MDR) is characterised as non-susceptibility to at least one agent in three or more antimicrobial categories, the analysis identified MDR in 46.4% of total *E. coli* isolates from LR and in 28.6% of isolates from SR[4].

Table 4: Table showing the percentage of MDR E. coli recovered from large ruminants and small ruminants. The MDR percentage was approximately 1.5 times more in large ruminants.

Animal group	Total Isolates	MDR isolates	MDR %
Large ruminants	153	71	46.4
Small ruminants	35	10	28.6

To further elucidate resistance patterns, a multi-class resistance profile was constructed. Figure 23 illustrates this profile for *E. coli* isolates from LR (n = 153) and SR (n = 35). Details are present in Annexe II - Table 23 and
Table 24.

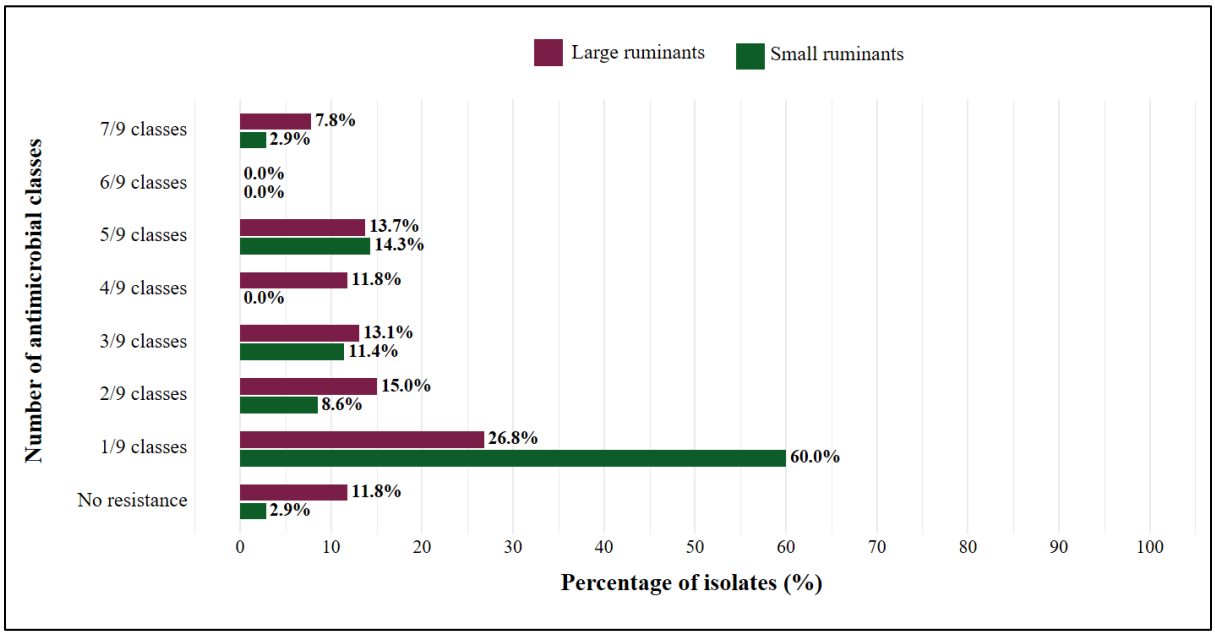

Figure 23: Multi-class resistance profile in E. coli for LR (Red; n = 153) and SR (Green; n = 35). Multi-class resistance was seen in as many as seven antibiotic classes.

Among non-MDR *E. coli* isolates, full susceptibility to all agents in the AST panel was uncommon, observed in only 2.9% of SR and 11.8% of LR isolates. Resistance confined to a **single antibiotic class** was more frequent, detected in 60.0% of SR and 26.8% of LR isolates. Resistance to **two classes** was identified in 8.6% of SR and 15.0% of LR isolates (Annexe II - Table 24).

Within the MDR population of *E. coli*, resistance to **three antimicrobial classes** was found in 11.4% of SR and 13.1% of LR isolates. The predominant three-class resistance phenotype in LR combined amoxicillin-enrofloxacin-gentamicin-norfloxacin (AMX-ENR-GEN-NOR). In SR, the predominant three-class resistance phenotype combined amoxicillin-enrofloxacin-gentamicin-norfloxacin (AMX-ENR-GEN-NOR).

Resistance to **four classes** was absent (0%) in SR isolates but present in 11.8% of LR isolates. The most common four-class phenotype was ampicillin-streptomycin-tetracycline-trimethoprim–sulfamethoxazole (AMP-STR-TCY-SXT).

Resistance to **five antibiotic classes** was observed at comparable levels: 14.3% in SR and 13.7% in LR isolates. The principal five-class resistance pattern in LR encompassed amoxicillin-ampicillin-chloramphenicol-doxycycline-enrofloxacin-tetracycline-trimethoprim–sulfamethoxazole (AMX-AMP-CHL-DOX-ENR-TCY-SXT) and in SR, it was amoxicillin-ampicillin-chloramphenicol-doxycycline-enrofloxacin-tetracycline-trimethoprim–sulfamethoxazole (AMX-AMP-CHL-DOX-ENR-TCY-SXT).

No isolates exhibited resistance to **six antimicrobial classes** concurrently. However, resistance to **seven classes** was detected in 2.9% of SR and 7.8% of LR isolates. The dominant seven-class resistance phenotypes in LR, included combinations such as ampicillin-chloramphenicol-doxycycline-enrofloxacin-gentamicin-nitrofurantoin-norfloxacin-streptomycin-tetracycline-trimethoprim–sulfamethoxazole (AMP-CHL-DOX-ENR-GEN-NIT-NOR-STR-TCY-SXT) and amoxicillin-ampicillin-cefoxitin-chloramphenicol-doxycycline-enrofloxacin-nitrofurantoin-tetracycline-trimethoprim–

sulfamethoxazole (AMX-AMP-FOX-CHL-DOX-ENR-NIT-TCY-SXT). In SR, the only isolate that showed resistance to seven-class, had the resistance phenotype ampicillin-chloramphenicol-doxycycline-enrofloxacin-gentamicin-nitrofurantoin-norfloxacin-streptomycin-tetracycline-trimethoprim–sulfamethoxazole (AMP-CHL-DOX-ENR-GEN-NIT-NOR-STR-TCY-SXT).

3.1.5.2 Salmonella spp. in livestock

In LR, MDR was observed in 58.8% of the total *Salmonella spp.* isolates and 23.8% in *Salmonella spp.* isolates from SR.

Table 5: Table showing the percentage of MDR Salmonella recovered from large ruminants and small ruminants. The MDR percentage was approximately 2.5 times more in large ruminants.

Animal group	Total Isolates	MDR isolates	MDR %
Large ruminants	51	30	58.8
Small ruminants	21	5	23.8

To further elucidate resistance patterns, a multi-class resistance profile was constructed. Figure 24 illustrates this profile for *Salmonella* spp. isolates from LR (n = 51) and SR (n = 21). The details are present in Annexe II - Table 23 and

Table 25.

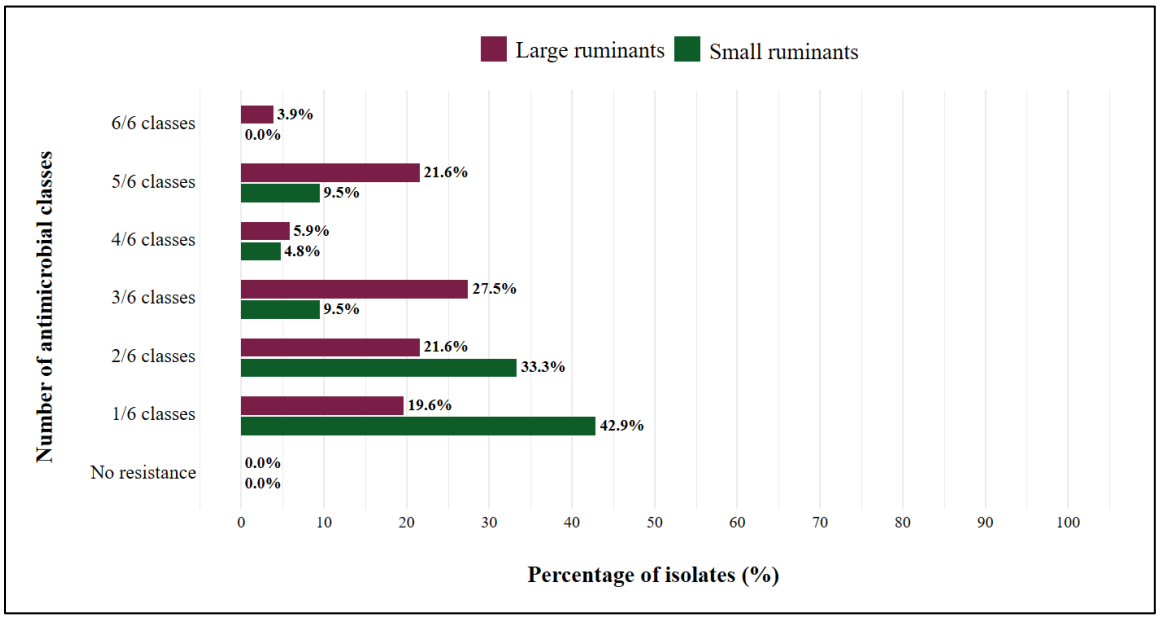

Figure 24: Multi-class resistance profile in Salmonella spp. for LR (n = 51) and SR (n = 21). Multi-class resistance was seen in as many as all six antibiotic classes tested.

Within the non-MDR group of *Salmonella* spp. isolates, none of the isolates were fully susceptible, they showed resistance to at least **one class** of antibiotics. Resistance to a single antibiotic class was observed in 42.9% of SR and 19.6% of LR. Resistance to **two classes** was identified in 33.3% of SR isolates and 21.6% of LR isolates.

Among the MDR *Salmonella* spp. isolates, resistance to **three classes** was found in 9.5% of SR and 27.5% in LR. Most of the LR isolates showed resistance to a combination of amoxicillin-ampicillin-doxycycline-enrofloxacin (AMX-AMP-DOX-ENR). Whereas the one SR isolate that showed resistance

to three class had the combination of amoxicillin-ampicillin-doxycycline-enrofloxacin (AMX-AMP-DOX-ENR).

Resistance to **four classes** was 4.8% in SR, 5.9% in LR. Most LR isolates showed resistance to a combination of ampicillin-chloramphenicol-doxycycline-tetracycline-trimethoprim–sulfamethoxazole (AMP-CHL-DOX-TCY-SXT). The one SR isolate showing resistance to four classes had the combination of amoxicillin-ampicillin-doxycycline-enrofloxacin-gentamicin (AMX-AMP-DOX-ENR-GEN).

Resistance to **five classes** of antibiotics was at 9.5% in SR and 21.6% in LR. The primary combination of drugs for LR included amoxicillin-ampicillin-chloramphenicol-doxycycline-enrofloxacin-tetracycline-trimethoprim–sulfamethoxazole (AMX-AMP-CHL-DOX-ENR-TCY-SXT). In SR, the isolates showed resistance to a combination of amoxicillin-ampicillin-chloramphenicol-doxycycline-enrofloxacin-tetracycline-trimethoprim–sulfamethoxazole (AMX-AMP-CHL-DOX-ENR-TCY-SXT).

No SR isolates showed resistance to **six classes** of antibiotics, yet 3.9% of LR isolates showed resistance to six classes. The primary combination of drugs was amoxicillin-ampicillin-chloramphenicol-doxycycline-enrofloxacin-gentamicin-tetracycline-trimethoprim–sulfamethoxazole (AMX-AMP-CHL-DOX-ENR-GEN-TCY-SXT).

3.1.5.3 S. aureus in livestock

In LR, MDR was observed in 41.4% of the total *S. aureus* isolates and 35.7% in *S. aureus* isolates from SR.

Table 6: Table showing the percentage of MDR S. aureus recovered from large ruminants and small ruminants. The MDR percentage was similar between the two categories.

Animal group	Total Isolates	MDR isolates	MDR %
Large ruminants	157	65	41.4
Small ruminants	14	5	35.7

To further elucidate resistance patterns in *S. aureus*, a multi-class resistance profile was constructed. Figure 25 illustrates this profile for from LR (n = 157) and SR (n = 14). The details are present in Annexe II - Table 22 and Table 26.

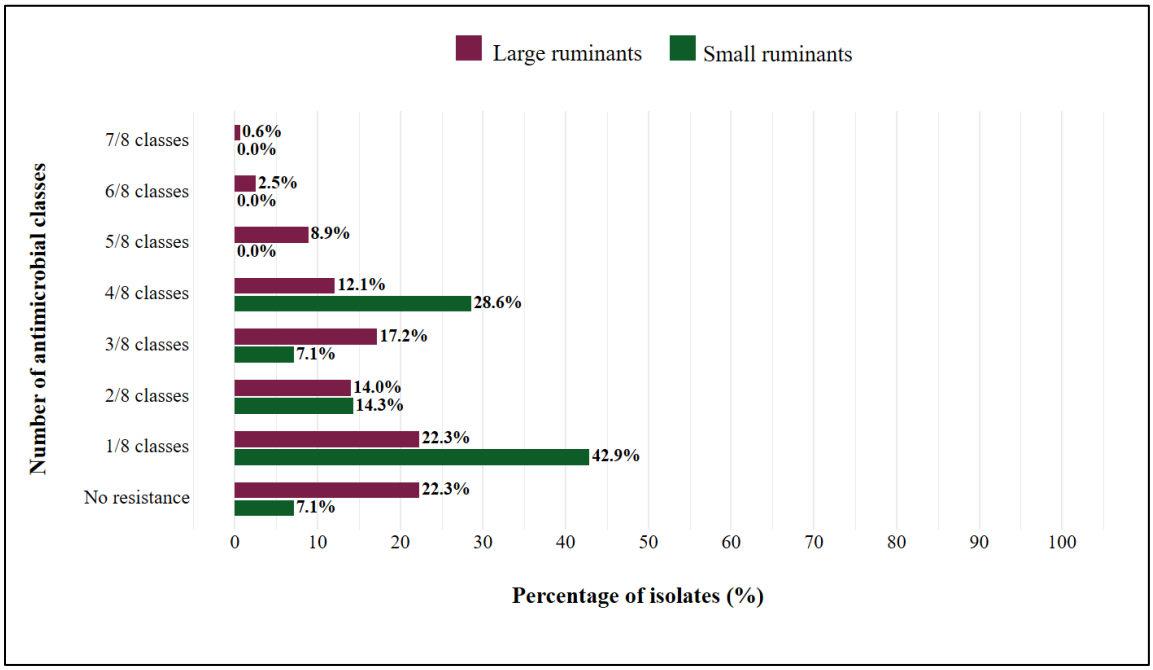

Figure 25: Multi-class resistance profile in Staphylococcus aureus for LR (n = 157) and SR (n = 14). Multi-class resistance was seen in as many as seven of the eight antibiotic classes tested.

Within the non-MDR group of *S. aureus* isolates, 7.1% of SR and 23.3 of LR isolates were fully susceptible. Resistance to **one class** of antibiotic was seen in 42.9% of SR and 22.3% of LR. Resistance to **two classes** was identified in 14.3% of SR isolates and 14% of LR isolates.

Among the MDR *S. aureus* isolates, resistance to **three classes** was found in 7.1% of SR and 17.2% in LR. Most of the LR isolates showed resistance to a combination of doxycycline-erythromycin-penicillin (DOX-ERY-PEN). Whereas the one SR isolate that showed resistance to three class had the Gentamicin-Penicillin-Trimethoprim–Sulfamethoxazole (GEN-PEN-SXT).

Resistance to **four classes** was 28.6% in SR, 12.1% in LR. Most LR isolates showed resistance to a combination of enrofloxacin-gentamicin-norfloxacin-oxytetracycline-penicillin (ENR-GEN-NOR-OXY-PEN). The only SR isolate showing resistance to four classes had the combination enrofloxacin-gentamicin-norfloxacin-oxytetracycline-penicillin (ENR-GEN-NOR-OXY-PEN).

Resistance to **five classes** of antibiotics was at 0.0% in SR and 8.9% in LR. The primary combination of drugs for LR included doxycycline-erythromycin-norfloxacin-penicillin-tetracycline-trimethoprim–sulfamethoxazole (DOX-ERY-NOR-PEN-TCY-SXT).

No SR isolates showed resistance to **six classes** of antibiotics, yet 2.5% of LR isolates showed resistance to six classes. The primary combination of drugs was chloramphenicol-doxycycline-erythromycin-gentamicin-norfloxacin-tetracycline-trimethoprim–sulfamethoxazole (CHL-DOX-ERY-GEN-NOR-TCY-SXT).

Resistance to **seven classes** of antibiotics was at 0.0% for SR and 0.6% for LR. Only one isolate of *S. aureus* was resistant to seven classes, with the antibiotic combination - chloramphenicol-doxycycline-erythromycin-gentamicin-norfloxacin-penicillin-tetracycline-trimethoprim–sulfamethoxazole (CHL-DOX-ERY-GEN-NOR-PEN-TCY-SXT).

3.2 Poultry

3.2.1 Bacterial recovery percentages in poultry

Figure 26 presents the recovery percentages of *E. coli*, *Salmonella* spp., and *S. aureus* from diseased poultry samples. From the 9,494 poultry samples, bacterial isolation was achieved for 1,318 *E. coli* isolates, corresponding to a minimum prevalence of 13.9% (95% CI: 13.2 - 14.58 %). Furthermore, 523 samples were positive for *Salmonella* spp. (5.5%, 95% CI: 5.06 - 5.98 %) and 369 for *S. aureus* (3.9%, 95% CI: 3.5 - 4.31 %).

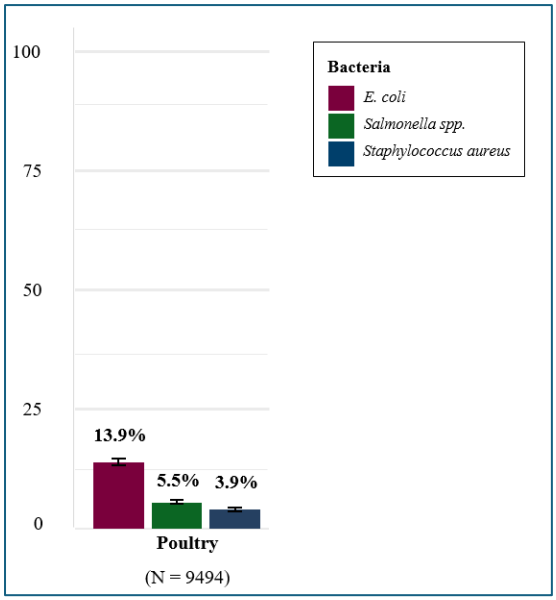

Figure 26: E. coli, Salmonella spp., and S. aureus isolation rates for poultry samples with the corresponding 95% confidence intervals.

The prevalence of *E. coli* was approximately three times as high in poultry as compared to other target pathogens (Table 7).

Table 7: Minimum prevalence of E. coli, Salmonella spp. and S. aureus pathogens in poultry with 95% confidence intervals.

Bacteria	Total samples (N)	Isolate (n)	Minimum prevalence [(n/N)*100]	95% Confidence interval
E. coli	9,494	1,318	13.9	13.2 - 14.58
Salmonella spp.	9,494	523	5.5	5.06 - 5.98
Staphylococcus aureus	9,494	369	3.9	3.5 - 4.31

Only a subset of the positive isolates underwent AST. The following flow chart presents a clear breakdown (Figure 27).

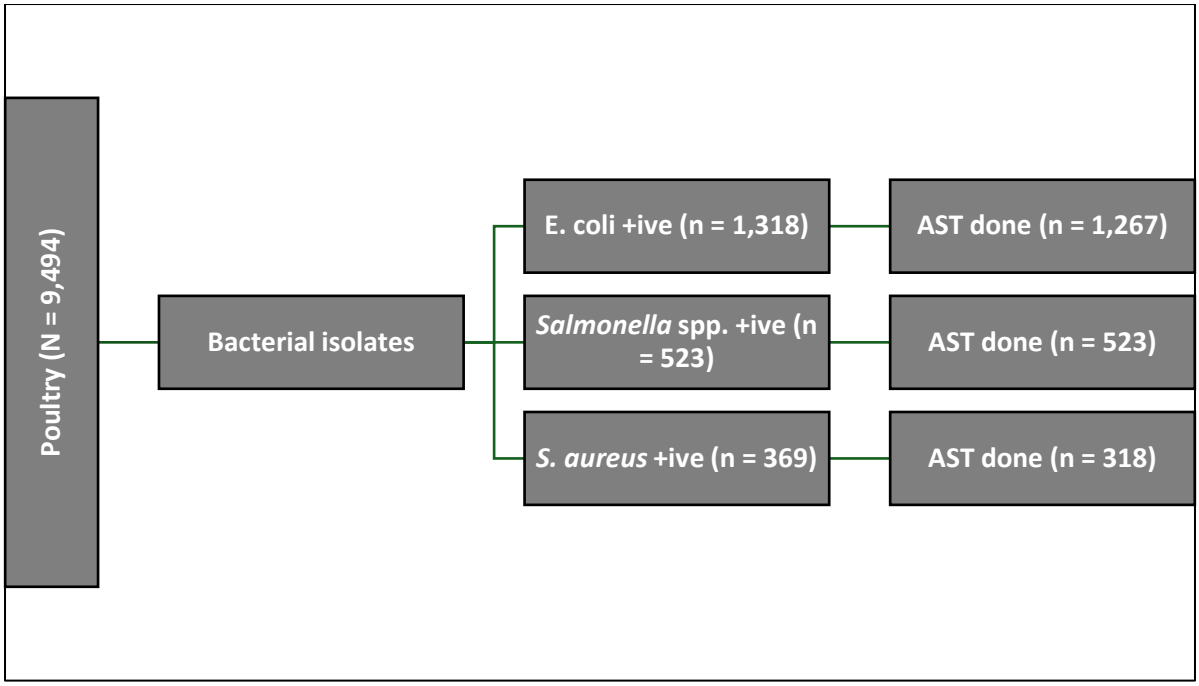

Figure 27: Flow chart shows the data for poultry. Not all samples submitted to sentinel sites underwent bacterial isolation, and neither did all bacterial isolates underwent AST.

3.2.2 Bacterial sample types

In Figure 28 the different sample types are presented that correspond to the bacterial isolates in the study. *E. coli* isolates were predominantly recovered from liver samples, which constituted 69.1% of the total. Other notable sources included lungs (7.9%), trachea (4.7%), and caecum (4.7%). The remaining isolates were distributed across various other tissues and environmental samples, each representing less than 3.3% of the collection. Their presence in extraintestinal sites is highly suggestive that these are potentially avian pathogenic *E. coli* (APEC) strains, possess virulence factors and responsible for acute to chronic and complicated disease syndromes in poultry. When resources allow, further characterisation of these isolates is warranted to better understand their epidemiology across poultry commodities.

Salmonella spp. isolates were also primarily sourced from the liver, accounting for 64.2% of specimens. Additional sample types were trachea (6.1%), drag swabs (5.5%), and eggs (4.6%). All other sample matrices, including cloaca, spleen, and heart, individually contributed less than 3% to the total isolate count.

For *S. aureus*, the liver was again the most common source (29.6%), though to a lesser extent than for other pathogens. Other sample matrices included the trachea (15.1%), yolk sac (13.2%), and bone marrow (10.1%). This indicates a broader distribution across systemic and localized infection sites within poultry (Annexe II, Table 17).

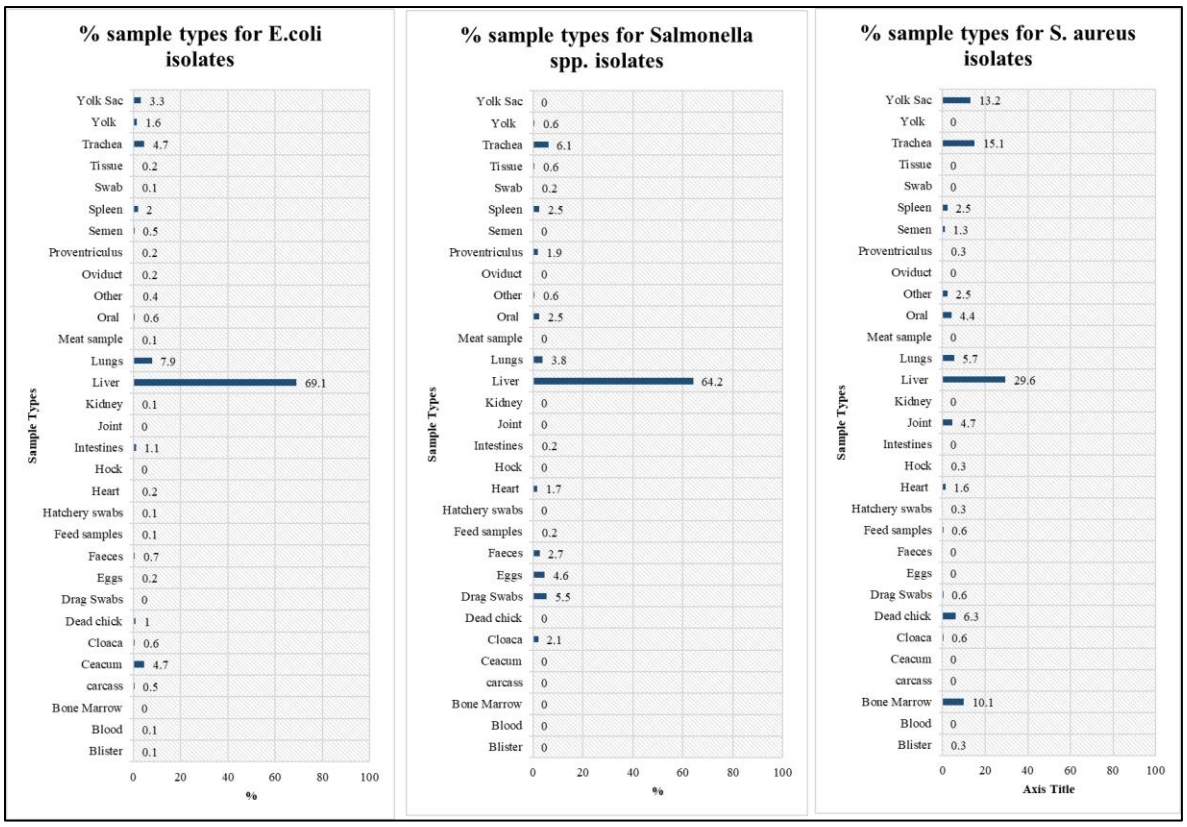

Figure 28: The bar charts detail the percentage of each sample type for E. coli, Salmonella spp. and S. aureus. The majority of the samples for all three target pathogens were collected post-mortem.

3.2.3 AMR phenotypes in poultry

3.2.3.1 Heat map analysis of AST patterns of bacterial isolates in poultry

The AST phenotypes of isolates for *E. coli*, *Salmonella* spp., and *S. aureus* were analysed. To synthesise and interpret the complex resistance profiles, the numerical resistance percentages for each pathogen-antibiotic combination were visualised using a hierarchical clustering heat map. This analytical tool employed the categorisation framework of the EUSR as previously described in the ruminant section. Additionally, percentage of resistance by individual antibiotics were annotated according to the WHO's MIA categorisation. The resultant visualisation reveals distinct clustering patterns, revealing resistance profiles across the studied pathogens.

3.2.3.2 E. coli isolates in poultry

The heat map presented in Figure 29 illustrates the AMR profile of *E. coli* isolates from poultry to fourteen antibiotics belonging to 9 classes. The overall resistance patterns observed for poultry was **high** to **extremely high** levels of resistance.

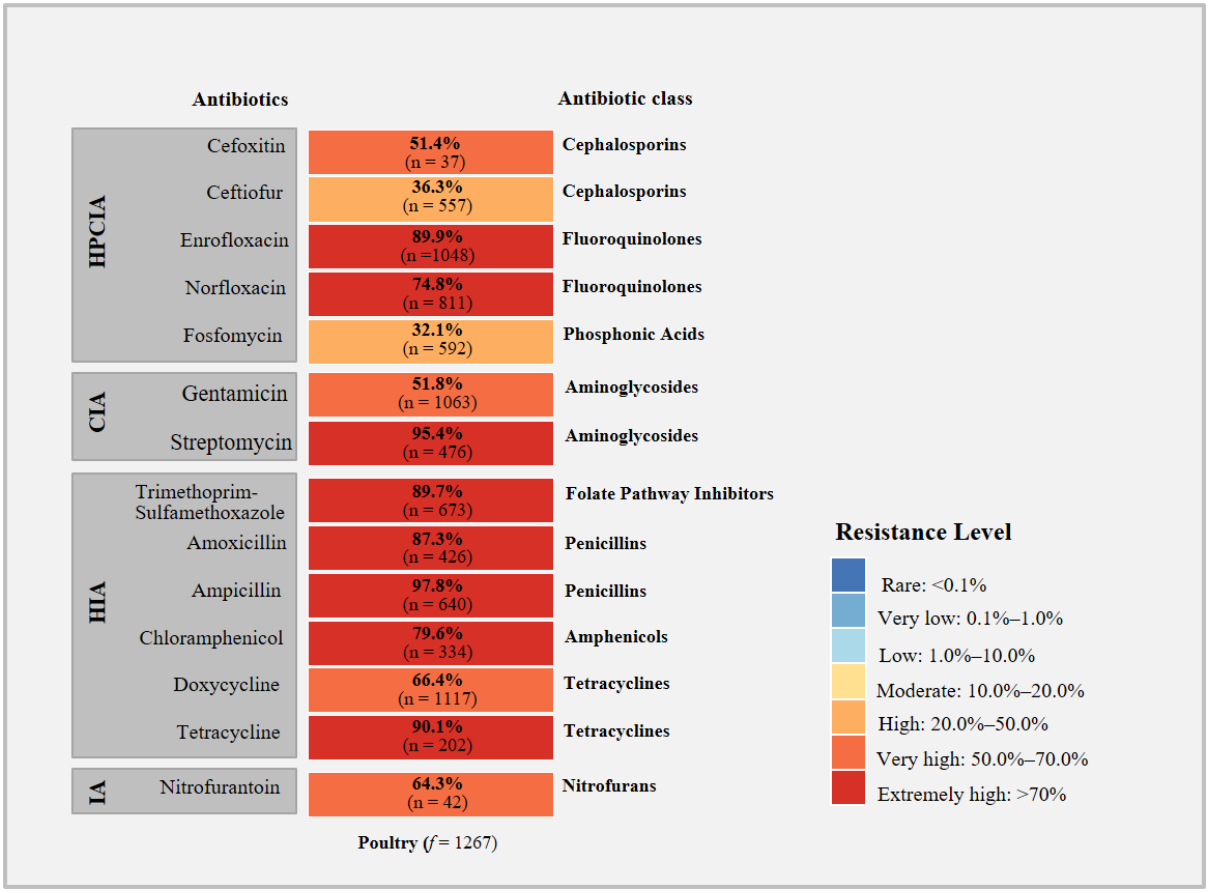

Figure 29: : Heat map of resistance percentages in E. coli isolated in poultry. The legend uses the EUSR's categorisation system. The antibiotics are classified based on WHO's MIA categories. 'n' in each cell refers to total count of isolates tested against each antibiotic demonstrating the need for harmonization of AST panels. 'f' refers to total isolates.

Analysis focused on the WHO categories of Highest Priority Critically Important Antimicrobials (HPCIAs) and Critically Important Antimicrobials (CIAs) revealed pronounced resistance:

1. **HPCIAs:** Resistance against norfloxacin (n = 811) and enrofloxacin (n = 1048) was **extremely high.** Cefoxitin (n = 37) showed **very high** resistance. Whereas, ceftiofur (n = 557) and fosfomycin (n = 591) showed **high** resistance.

2. **CIAs:** Streptomycin demonstrated **extremely high** resistance (n = 476). While resistance to gentamicin was **very high** (n = 1063).

3.2.3.3 *Salmonella spp. isolates in poultry*

The heat map presented in Figure 30 illustrates the AMR profile of *Salmonella* spp. isolates from poultry against nine antibiotics. The overall resistance patterns observed was again notably similar, with the majority of antibiotics displaying **high** to **extremely high** levels of resistance.

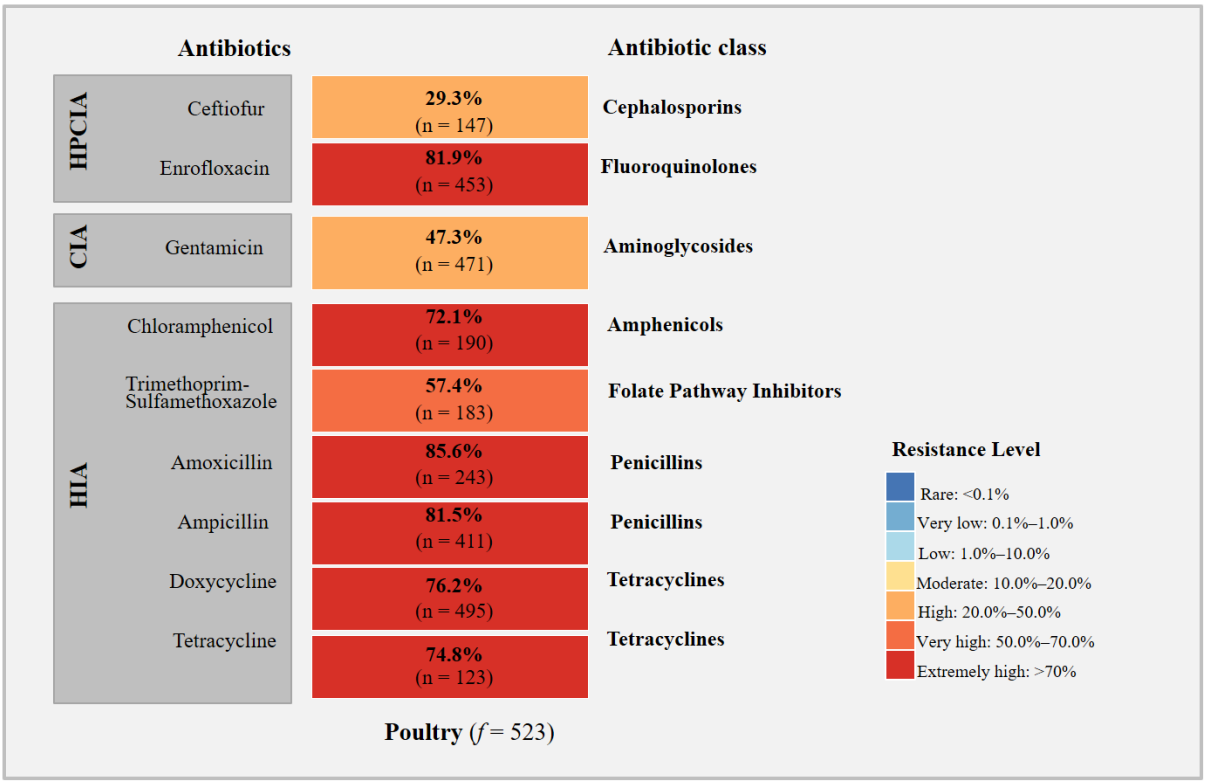

Figure 30: Heat map of resistance percentages in Salmonella spp. isolated in poultry. The legend uses the EUSR's categorisation system. The antibiotics are classified based on WHO's MIA categories. 'n' in each cell refers to total count of isolates tested against each antibiotic demonstrating the need for harmonization of AST panels. 'f' refers to total isolates.

Analysis focused on the WHO categories of HPCIAs and CIAs revealed pronounced resistance:

1. **HPCIAs:** Resistance to enrofloxacin was **extremely high** in (n = 543) and **high** in ceftiofur (n = 147)
2. **CIAs:** Resistance to gentamicin was **high** (n = 471).

3.2.3.4 *Staphylococcus aureus isolates in poultry*

The heat map presented in Figure 31 illustrates the AMR profile of *S. aureus*. isolates from poultry to twelve antibiotics belonging to 9 classes. The overall resistance patterns observed was yet again comparable to both *E. coli* and *Salmonella* spp., with the majority of antibiotics displaying **high** to **extremely high** levels of resistance.

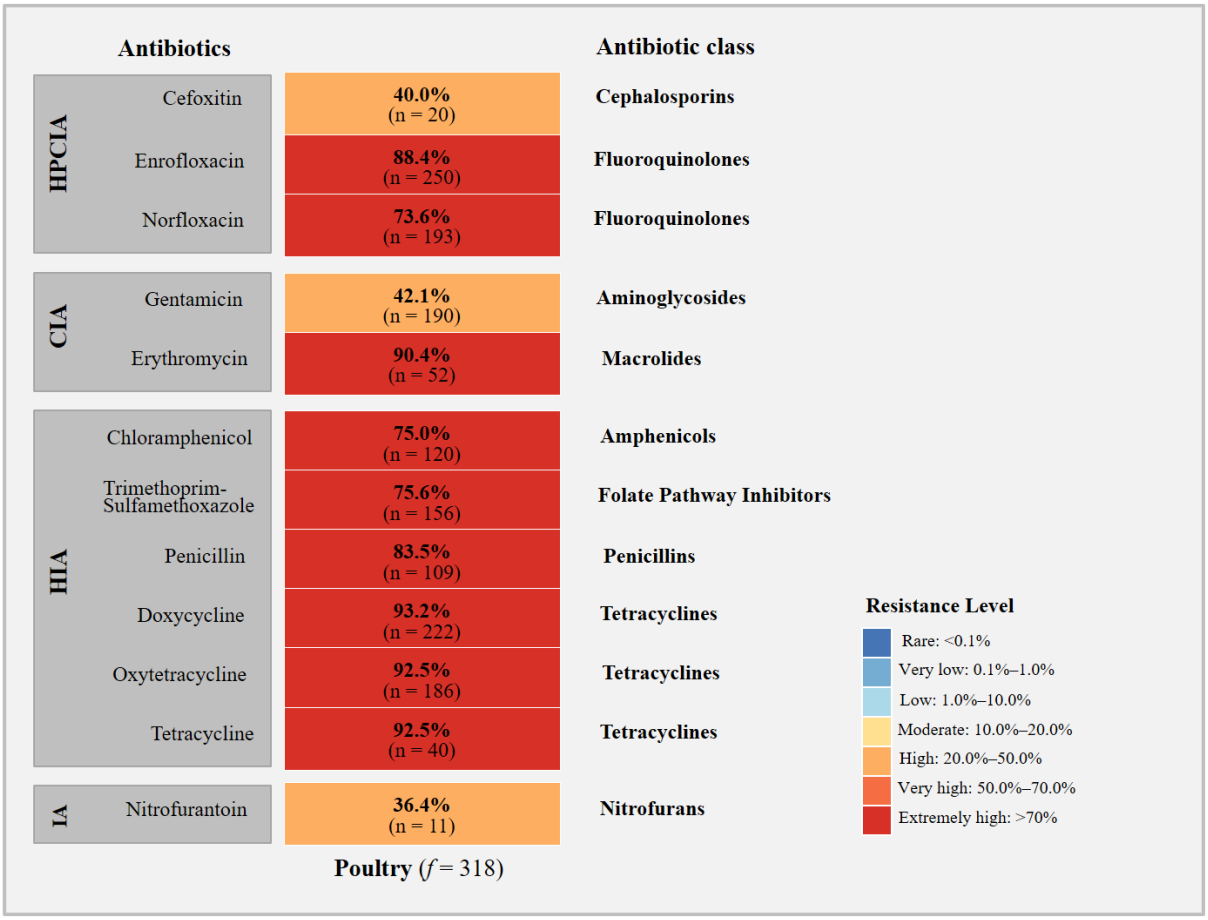

Figure 31: Heat map of resistance percentages in S. aureus isolated in poultry. The legend uses the EUSR's categorisation system. The antibiotics are classified based on WHO's MIA categories. 'n' in each cell refers to total count of isolates tested against each antibiotic demonstrating the need for harmonization of AST panels. 'f' refers to total isolates.

Analysis focused on the WHO categories of HPCIAs and CIAs revealed pronounced resistance:

1. **HPCIAs:** Resistance to norfloxacin (n = 193) as well as enrofloxacin (n = 250) was **extremely high**. Whereas resistance was **high** to cefoxitin (n = 20).
2. **CIAs:** Resistance to erythromycin was **extremely high** (n = 52), whereas in gentamicin resistance was **high** (n = 190).

3.2.4 AMR patterns based on WHO priority classification of bacterial isolates in poultry

3.2.4.1 E. coli AMR in poultry

Figure 32 illustrates the AMR profile of 1,267 *E. coli* isolates recovered from diseased poultry. Antibiotics are categorised according to the WHO classifications of HPCIA, CIA, HIA, and IA. Resistance varied substantially, ranging from 32.1% to 97.8% (Annex II - Table 20).

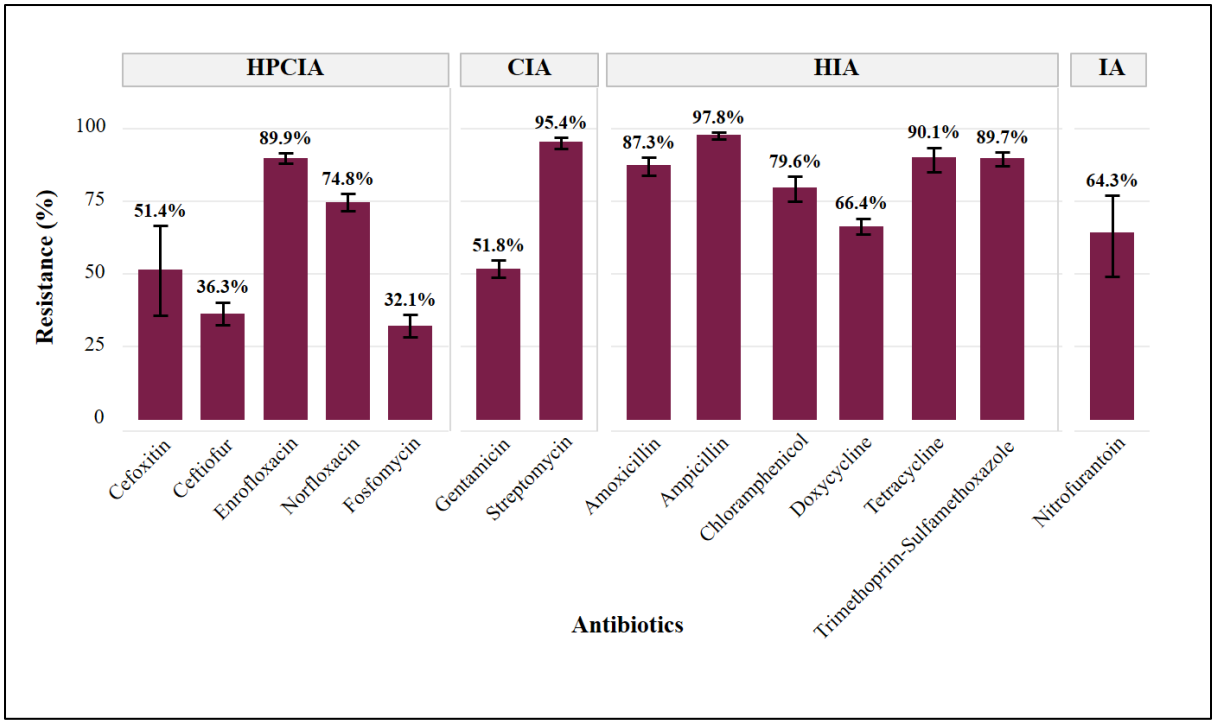

Figure 32: AST pattern of E. coli isolates in poultry (n = 1,267) by antibiotics and WHO categorisation.

In the HPCIA category, cephalosporin and fluoroquinolones showed variable resistance: cefoxitin 51.4% (95% CI: 35.9 - 66.6%), ceftiofur 36.3% (95% CI: 32.4 - 40.3), enrofloxacin 89.9% (95% CI: 87.9 - 91.6%), norfloxacin 74.8% (95% CI: 71.7 - 77.7%) and fosfomycin 32.1% (95% CI: 28.5 - 36%)

For CIA agents, aminoglycosides showed distinct resistance: gentamicin demonstrated 51.8% resistance (95% CI: 48.8 - 54.8%) and streptomycin 95.4% (95% CI: 93.1 - 96.9%).

Among HIA compounds, amoxicillin showed resistance at 87.3% (95% CI: 83.8 - 90.2%), ampicillin at 97.8% (95% CI: 96.4 - 98.7), chloramphenicol at 79.6% (95% CI: 75 - 83.6%), doxycycline at 66.4% (95% CI: 63.6 - 69.1%), tetracycline at 90.1% (95% CI: 85.2 - 93.5%) and trimethoprim-sulphamethoxazole at 89.7% (95% CI: 87.2 - 91.8%).

Of the IA compounds, 64.3% resistance was observed against Nitrofurantoin (95% CI: 49.2 - 77%).

3.2.4.2 Salmonella spp. AMR in poultry

Figure 33 delineates the AMR profile for a panel of 523 *Salmonella* spp. isolates recovered from diseased poultry. A substantial variation in resistance prevalence was observed across the tested antimicrobial agents, ranging from 29.3% to 85.6% (Annexe II - Table 21).

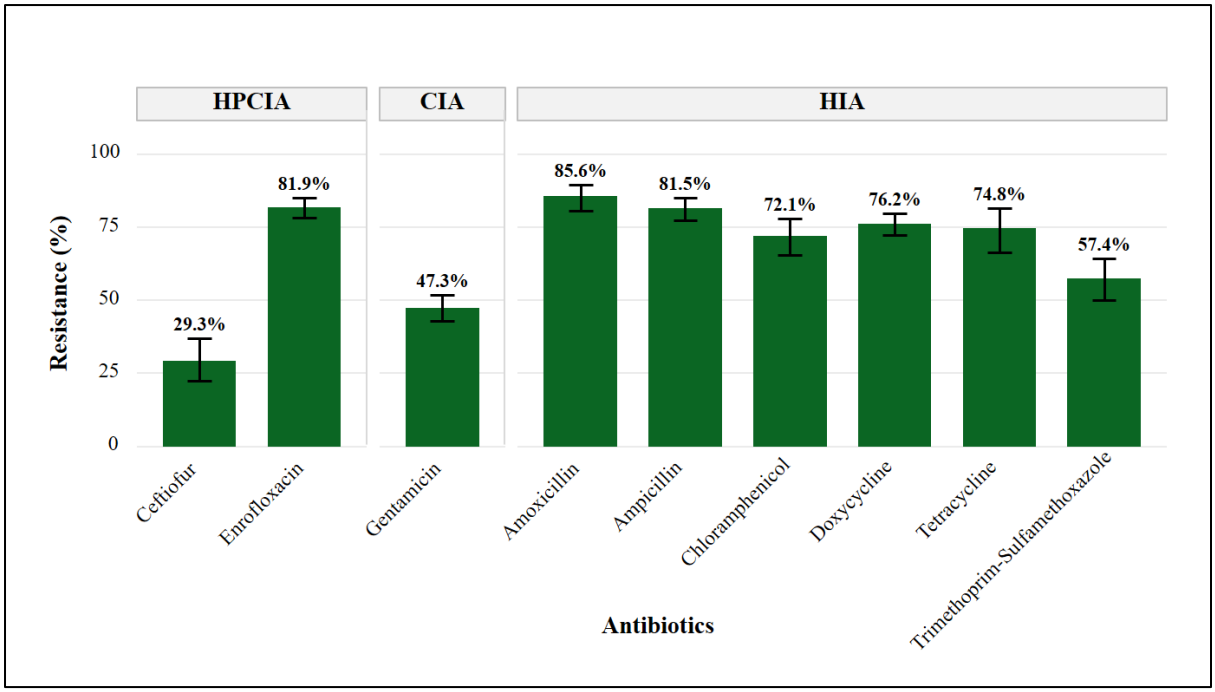

Figure 33: AST pattern of Salmonella spp. isolates in poultry (n = 523) by antibiotics and WHO categorisation.

Amongst the concerning agents classified as HPCIAs, resistance to enrofloxacin was recorded at 81.9% (95% CI: 78.1 - 85.2%) and ceftiofur was 29.3% (95% CI: 22.5 - 37.1).

For the CIA agent gentamicin, a resistance prevalence of 47.3% (95% CI: 42.9 - 51.9%) was demonstrated.

Among the HIA compounds, resistance was notably high for several agents: amoxicillin at 85.6% (95% CI: 80.6 - 89.5%), ampicillin at 81.5% (95% CI: 77.5 - 85%), and chloramphenicol at 72.1% (95% CI: 65.3 - 78%). Elevated resistance was also observed for tetracycline at 74.8% (95% CI: 66.5 - 81.6%), doxycycline at 76.2% (95% CI: 72.2 - 79.7%). Lower though still important resistance at 57.4%, was identified for trimethoprim-sulphamethoxazole (95% CI: 50.1 - 64.3%).

3.2.4.3 Staphylococcus aureus AMR in poultry

Figure 34 illustrates the AMR characteristics of 318 *Staphylococcus aureus* isolates derived from clinical poultry cases. The prevalence of resistance demonstrated a wide variation, extending from 36.4% to 93.2% among the evaluated antimicrobials (Annex II - Table 22)

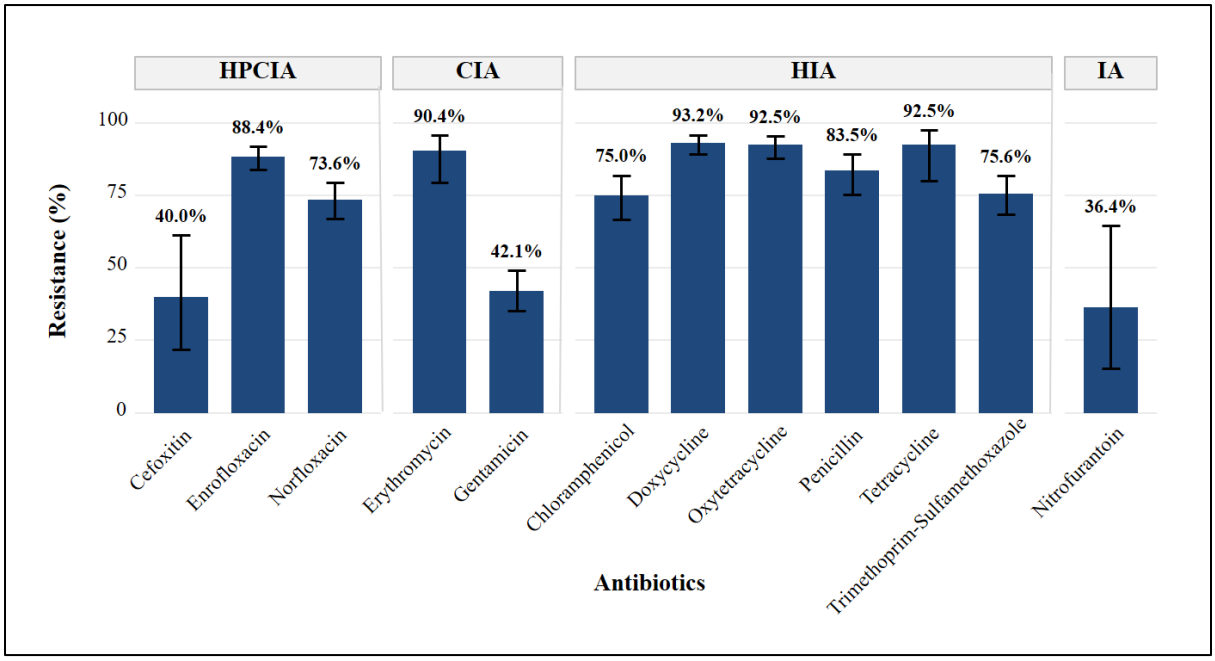

Figure 34: AST pattern of Staphylococcus aureus isolates in poultry (n = 318) by antibiotics and WHO categorisation

In the HPCIA classification, the resistance level for cefoxitin was measured at 40.0% (95% CI: 21.9 - 61.3%). Conversely, resistance to the fluoroquinolones was markedly greater, with values of 88.4% (95% CI: 83.8 - 91.8%) for enrofloxacin and 73.6% (95% CI: 66.9 - 79.3%) for norfloxacin.

For CIA agents, erythromycin resistance stood at 90.4% (95% CI: 79.4 - 95.8%), whereas gentamicin resistance was lower at 42.1% (95% CI: 35.3 - 49.2%).

Concerning HIA compounds all antibiotics showed extremely high resistance. Resistance against doxycycline was at 93.2% (95% CI: 89.2 - 95.9%) and oxytetracycline at 92.5% (95% CI: 87.8 - 95.5%). Resistance to tetracycline was 92.5% (95% CI: 80.1 - 97.4%), and to penicillin 83.5% (95% CI: 75.4 - 89.3%). Extremely high resistance levels were documented for trimethoprim-sulfamethoxazole at 75.6% (95% CI: 68.3 - 81.7%) and for chloramphenicol at 75% (95% CI: 66.6 - 81.9%).

Amongst HI agents, resistance to nitrofurantoin was 36.4% (95% CI:15.2 - 64.6).

3.2.5 Multi-class resistance and MDR profile of bacterial isolates in poultry

3.2.5.1 E. coli in poultry

The analysis identified MDR in 78.8% of total *E. coli* isolates from poultry (Table 8).

Table 8: MDR percentage in E. coli isolates of poultry.

Animal group	Total Isolates	MDR isolates	MDR %
Poultry	1267	998	78.8

To further elucidate resistance patterns, a multi-class resistance profile was constructed. The results revealed 314 different combinations of antibiotics. Figure 35 illustrates this profile for *E. coli* isolates from poultry.

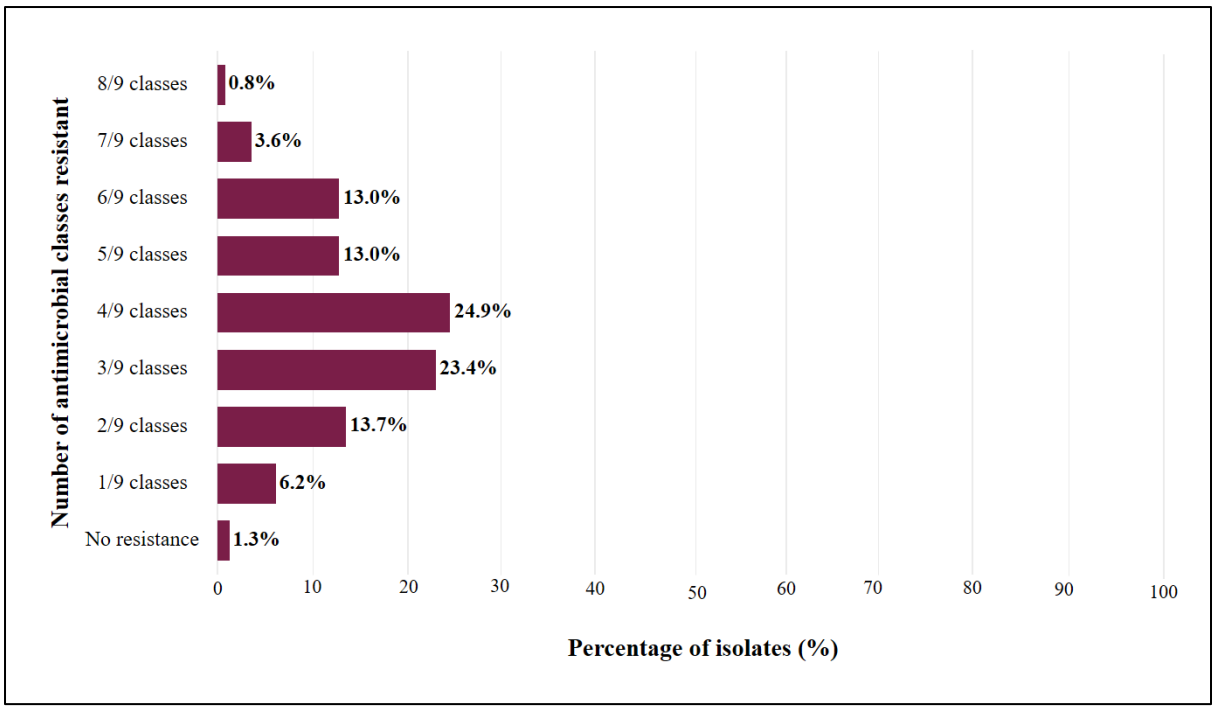

Figure 35: Multi-class resistance profile in E. coli for poultry (n = 1267). Multi-class resistance was seen in as many as eight of the nine antibiotic classes.

Among non-MDR *E. coli* isolates, full susceptibility to all agents in the AST panel was uncommon, observed in only 1.3% of poultry isolates. Resistance confined to a **single antibiotic class** was more frequent, detected in 6.2% of poultry isolates. Resistance to **two classes** was identified in 13.7% of poultry isolates (Annexe II - Table 23).

Within the MDR population of *E. coli* isolates in poultry, resistance to **three antimicrobial classes** was found in 23.4% of poultry isolates. The predominant three-class resistance phenotype in poultry combined doxycycline-enrofloxacin-norfloxacin-trimethoprim-sulfamethoxazole (DOX-ENR-NOR-SXT).

Resistance to **four classes** was found in 24.9% in poultry isolates. The most common four-class phenotype was doxycycline-enrofloxacin-gentamicin-norfloxacin-trimethoprim-sulfamethoxazole (DOX-ENR-GEN-NOR-SXT).

Resistance to **five antibiotic classes** was observed at 13% in poultry isolates. The principal five-class resistance pattern in poultry encompassed amoxicillin-ampicillin-chloramphenicol-doxycycline-enrofloxacin-streptomycin-trimethoprim-sulfamethoxazole (AMX-AMP-CHL-DOX-ENR-STR-SXT).

Resistance to **six antimicrobial classes** observed at comparable levels, 13% in poultry isolates. The principal six-class resistance pattern in poultry encompassed ampicillin-chloramphenicol-doxycycline-enrofloxacin-streptomycin-tetracycline-trimethoprim-sulfamethoxazole (AMP-CHL-DOX-ENR-STR-TCY-SXT).

Resistance to **seven classes** was detected in 3.6% of poultry isolates. The dominant seven-class resistance phenotype was amoxicillin-ampicillin-chloramphenicol-doxycycline-enrofloxacin-gentamicin-norfloxacin-streptomycin-trimethoprim-sulfamethoxazole (AMX-AMP-CHL-DOX-ENR-GEN-NOR-STR-SXT).

Lastly, resistance to **eight classes** was detected in 0.8% of poultry isolates. The dominant eight-class resistance phenotype was ampicillin-chloramphenicol-doxycycline-enrofloxacin-gentamicin-nitrofurantoin-norfloxacin-streptomycin-tetracycline-trimethoprim-sulfamethoxazole (AMP-CHL-DOX-ENR-GEN-NIT-NOR-STR-TCY-SXT)

The details of these antibiotic class combination are present in Annexe II - Table 24.

3.2.5.2 Salmonella spp. in poultry

In poultry, MDR was observed in 70% of the total *Salmonella spp.* isolates.

Table 9: MDR percentage in Salmonella spp. isolates in Livestock.

Animal group	Total Isolates	MDR isolates	MDR %
Poultry	523	366	70

To further elucidate resistance patterns, a multi-class resistance profile was constructed. The results revealed 117 different combinations of antibiotics. Figure 36 illustrates this profile for *Salmonella spp.* isolates from poultry.

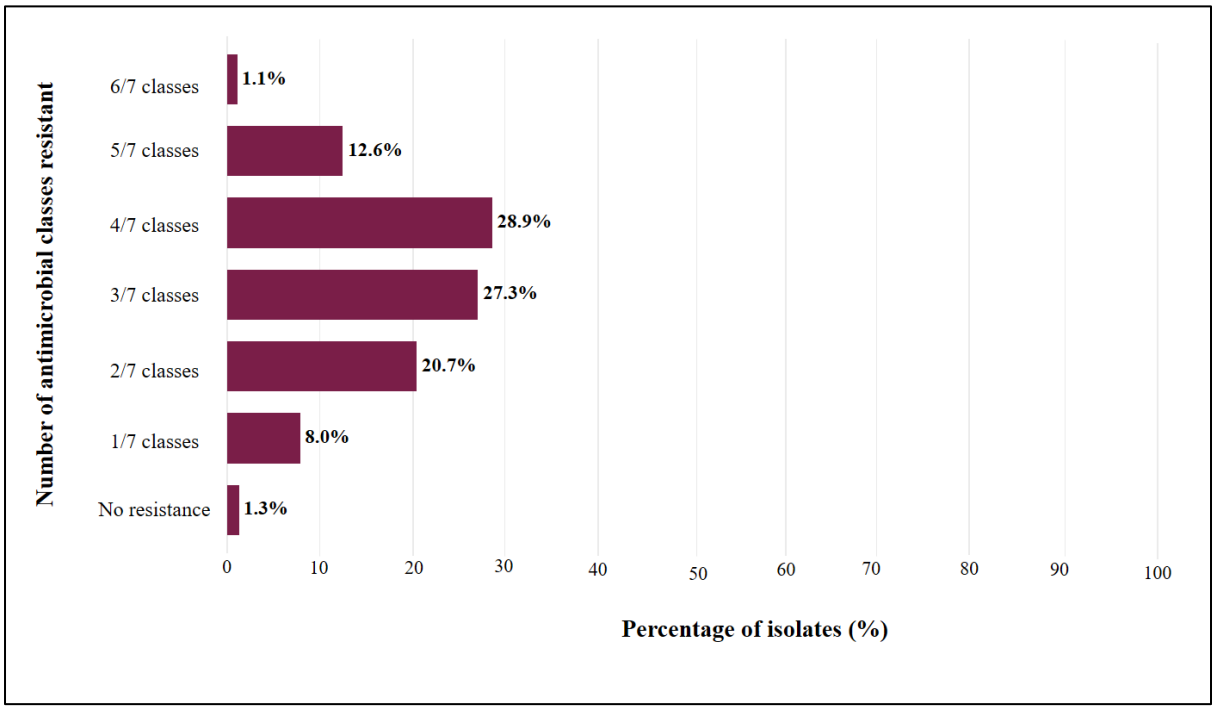

Figure 36:Multi-class resistance profile in Salmonella spp. for poultry. Multi-class resistance was seen in as many as six of the seven antibiotic classes tested.

Among non-MDR *Salmonella spp.* isolates, full susceptibility to all agents in the AST panel was uncommon, observed in only 1.3% of poultry isolates. Resistance confined to a **single antibiotic class** was more frequent, detected in 8.0% of poultry isolates. Resistance to **two classes** was identified in 20.7% of poultry isolates (Annexe II - Table 23).

Within the MDR population of *Salmonella spp.* isolates in poultry, resistance to **three antimicrobial classes** was found in 27.3% of poultry isolates. The predominant three-class resistance phenotype in poultry combined amoxicillin-ampicillin-doxycycline-enrofloxacin (AMX-AMP-DOX-ENR).

Resistance to **four classes** was found in 28.9% in poultry isolates. The most common four-class phenotype was ampicillin-doxycycline-enrofloxacin-gentamicin (AMP-DOX-ENR-GEN).

Resistance to **five antibiotic classes** was observed at 12.6% in poultry isolates. The principal five-class resistance pattern in poultry encompassed amoxicillin-ampicillin-chloramphenicol-doxycycline-enrofloxacin-gentamicin (AMX-AMP-CHL-DOX-ENR-GEN).

Lastly, resistance to **six antimicrobial classes** observed at comparable levels, 1.1% in poultry isolates. The principal six-class resistance pattern in poultry encompassed amoxicillin-ampicillin-chloramphenicol-doxycycline-enrofloxacin-tetracycline-trimethoprim-sulfamethoxazole (AMX-AMP-CHL-DOX-ENR-TCY-SXT).

The details of these antibiotic class combination are present in Annex II - Table 25.

3.2.5.3 Staphylococcus aureus in poultry

In poultry, MDR was observed in 63.8% of the total *S. aureus* isolates.

Table 10: MDR percentage of S. aureus isolates in poultry.

Animal group	Total Isolates	MDR isolates	MDR %
Poultry	318	203	63.8

To further elucidate resistance patterns, a multi-class resistance profile was constructed. The results revealed 65 different combinations of antibiotics. Figure 37 illustrates this profile for *S. aureus* isolates from poultry.

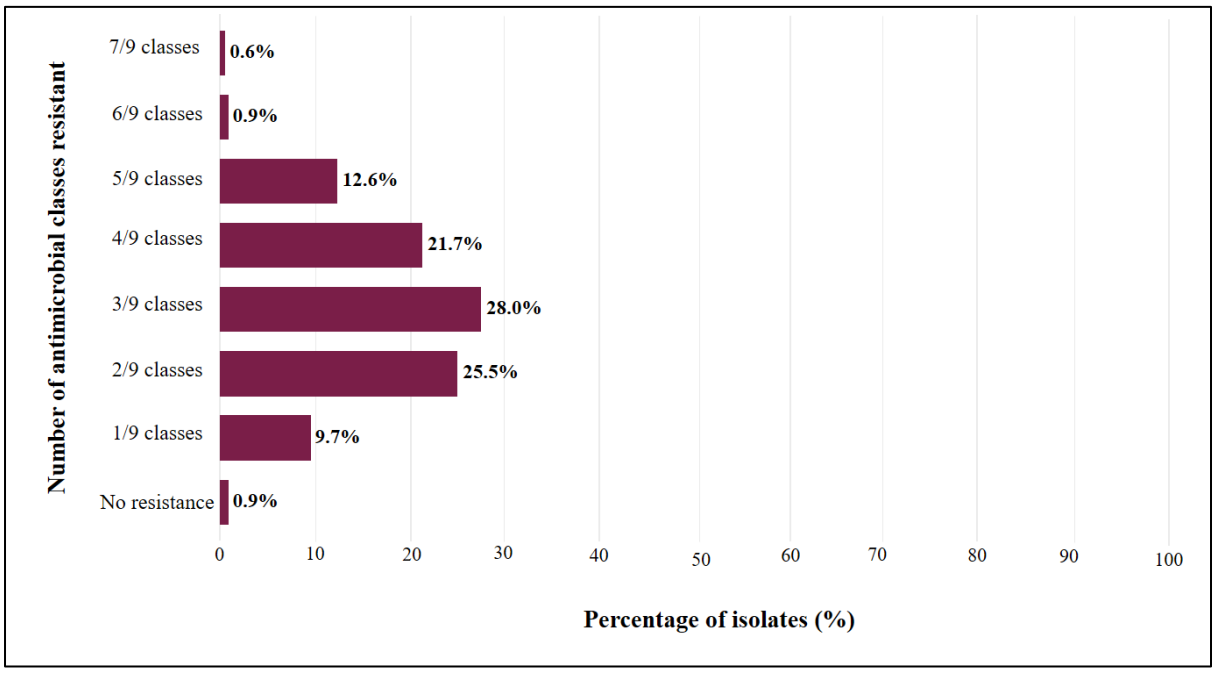

Figure 37: Multi-class resistance profile in S. aureus for poultry. Multi-class resistance was seen in as many as seven of the nine antibiotic classes tested.

Among non-MDR *S. aureus* isolates, full susceptibility to all agents in the AST panel was uncommon, observed in only 0.9% of poultry isolates. Resistance confined to a **single antibiotic class** was more frequent, detected in 9.7% of poultry isolates. Resistance to **two classes** was identified in 25.5% of poultry isolates (Annexe II - Table 23).

Within the MDR population of *S. aureus* isolates in poultry, resistance to **three antimicrobial classes** was found in 28.0% of poultry isolates. The predominant three-class resistance phenotype in poultry combined enrofloxacin-oxytetracycline-penicillin (ENR-OXY-PEN).

Resistance to four classes was found in 21.7% in poultry isolates. The most common **four-class** phenotype was chloramphenicol-enrofloxacin-tetracycline-trimethoprim-sulfamethoxazole (CHL-ENR-TCY-SXT).

Resistance to five antibiotic classes was observed at 12.6% in poultry isolates. The principal **five-class** resistance pattern in poultry encompassed chloramphenicol-doxycycline-enrofloxacin-norfloxacin-oxytetracycline-trimethoprim-sulfamethoxazole (CHL-DOX-ENR-NOR-OXY-SXT).

Resistance to **six antimicrobial classes** observed at comparable levels, 0.9% in poultry isolates. The principal six-class resistance pattern in poultry encompassed chloramphenicol-doxycycline-enrofloxacin-gentamicin-norfloxacin-oxytetracycline-trimethoprim-sulfamethoxazole (CHL-DOX-ENR-GEN-NOR-OXY-SXT).

Lastly, resistance to **seven antimicrobial classes** observed at comparable levels, 0.6% in poultry isolates. The principal seven-class resistance pattern in poultry chloramphenicol-doxycycline-erythromycin-gentamicin-norfloxacin-penicillin-tetracycline-trimethoprim-sulfamethoxazole (CHL-DOX-ERY-GEN-NOR-PEN-TCY-SXT).

The details of these antibiotic class combination are present in Annexe II - Table 26.

4 Discussion

The findings from this initial national passive AMR surveillance for diseased FPAs in Pakistan provide a critical snapshot of the therapeutic challenges facing the veterinary sector. Importantly, this study establishes a national baseline of AMR data from the diseased terrestrial animal domain, that informs animal health decision-making for the preservation of the remaining antimicrobials efficacious for the treatment of diseases in animals. In parallel, through this FFCGP supported programme, it operationalised and strengthened a national veterinary diagnostic and reporting network, laying the foundation for sustained and expanded AMR surveillance in diseased animals beyond the Grant.

The high prevalence of resistance, particularly to WHO's CIAs, must be interpreted within the appropriate clinical, epidemiological, and local production context. Passive surveillance in diseased animals preferentially captures pathogens that have persisted or emerged following empirical on-farm antimicrobial treatment. As such, the data do not represent the broader commensal or general bacterial population, but rather a selected subset of organisms that have survived initial therapeutic pressure, thereby explaining the extremely high-level resistance and diverse phenotypic profiles observed. Viewed through this lens, the findings shift from a public health alarm to a clinically actionable veterinary tool, providing evidence to informed and targeted treatment practices and awareness towards the preservation of the efficacy of antimicrobials.

The integration of metadata such as sample type further strengthens interpretation by linking AMR findings to specific syndromes that prompt diagnostic submission. This contextualisation reveals that the most concerning resistance profiles are closely associated with disease conditions reflecting treatment failure and antimicrobial exposure histories within livestock production systems.[15]. Beyond its immediate findings, the programme demonstrates that existing veterinary diagnostic laboratories can continue to generate AMR data and optimise current structures and resources; however, long-term sustainability will require further strengthening of harmonised laboratory methods (standard AST panel), quality assurance, and standardized data management (reporting of both raw and interpreted values) to ensure comparability and utility for national and global reporting.

4.1 E. coli

The high proportion of *E. coli* recovered from poultry liver (69.1%) and bovine milk (81.7% in LR) reflect its importance in various diseases. In poultry, liver isolates signify the presence of colisepticemia (APEC-associated_, typically a sub-acute condition suggesting failure of initial flock management or therapy. The concurrent high resistance to fluoroquinolones (e.g., enrofloxacin, 89.9% in poultry) and beta-lactams aligns with patterns of use and confirms their diminished value as empirical choices for such severe cases. Improved on-farm management practices would reduce the need for antibiotics and are an important component of AMS programmes to reduce AMU in farms animals.

[15] Harun, A. B., Khatri, B., Karim, M. R., Azmi, S., Adnan, M., & Hassan, M. M. (2024). Phenotypic and genotypic patterns of antimicrobial resistance in livestock and poultry in South Asia: A systematic review and meta-analysis. Food Control, 164, 110575.

In dairy animals, the overwhelming association with mastitic milk directly links the pathogen to one of the costliest diseases in Pakistan's dairy sector, where *E. coli* is a recognized environmental mastitis agent[16]. The high resistance observed correlates strongly with documented practices in the sector. Studies indicate a high prevalence of unregulated AMU, with dairy farmers frequently engaging in non-prescribed use, early discontinuation of therapy, and sharing of antibiotics—practices that create sustained selective pressure for resistance[17]. This pressure is evident in findings from Pakistani buffalo mastitis milk, where *E. coli* isolates show high resistance to ampicillin (68%) and tetracycline (81%), and a significant proportion carry extended-spectrum beta-lactamase (ESBL) genes like blaCTX-M-1[18].

Table 11: Key E. coli AMR Findings in Context

Parameter	**Finding in This Passive Surveillance**	**Interpretation in Light of Sample Type & Regional Context**	**Reference**
Primary Sample Source (Poultry)	Liver (69.1%)	Indicates systemic infection; isolates likely to represent cases after empirical farm treatment, explaining extreme resistance.	
Primary Sample Source (LR)	Milk (81.7%)	Confirms high proportion of samples from clinical mastitis cases; high AMR reflects documented treatment pressure in dairy sector.	Farhan et al., (2024)[17] Ghumman et al., (2025)[16]
Key AMR Pattern	Extremely high resistance to amoxicillin, enrofloxacin, tetracycline.	Aligns with reported use of these drugs for prophylaxis and therapy. Resistance is a direct outcome of this use pattern	Abro et al., (2024)[18]
Driver of Resistance	Selection from pre-treatment.	Linked to practices of self-medication, non-adherence to dosage, and sharing of	Farhan et al., (2024)[17]

[16] Ghumman, N. Z., Bruce, M., Barbosa, A. D., Ijaz, M., Peng, J., & Gogoi-Tiwari, J. (2025). Bovine mastitis and antimicrobial resistance in Pakistan's dairy sector: current status and future prospects. Veterinary research communications, 50(1), 31. https://doi.org/10.1007/s11259-025-10951-1

[17] Farhan, M., Awan, N., Kanwal, A. et al. Dairy farmers' levels of awareness of antibiotic use in livestock farming in Pakistan. Humanit Soc Sci Commun 11, 165 (2024). https://doi.org/10.1057/s41599-023-02518-9

[18] Sarang Mazhar Abro, Jam Kashif Sahito, Abdul Ahad Soomro, Amjad Hussain Mirani, Muhammad Azhar Memon, Nazeer Hussain Kalhoro, Detection of extended-spectrum beta-lactamase genes among Escherichia coli isolates of buffalo mastitis milk, Ecological Genetics and Genomics, Volume 33, 2024, 100297, ISSN 2405-9854, https://doi.org/10.1016/j.egg.2024.100297.

		antibiotics among farmers	

4.2 *Salmonella* spp.

The data from passive surveillance for *Salmonella* spp. provides a clinically and epidemiologically coherent picture. The pattern of sample origins is particularly revealing in poultry, the pathogen was primarily recovered from the liver (64.2%), while in ruminants, it was isolated from a diverse range of systemic sites, including milk (25.5% in Large Ruminants), lungs, liver, and heart. This distribution is a classical indicator of invasive, systemic disease—such as fowl typhoid in poultry or septicemic salmonellosis in cattle—where the bacterium has breached the intestinal barrier and disseminated to internal organs[19].

This pattern underscores a significant surveillance gap. While this passive system detects the threat, assessments of Pakistan's National Action Plan (NAP) on AMR note the need for more scalable, sustained surveillance in livestock to fully understand and manage such foodborne risks[20] and most notably to inform treatment guidelines supporting appropriate use of antimicrobials in animals. The data acts as a sentinel, detecting high-risk MDR zoonotic pathogens that have successfully caused disease in animals and pose a subsequent threat to humans through the food chain.

The AMR profile of these isolates is severe and aligns with a growing regional crisis. The extremely high resistance to the fluoroquinolone enrofloxacin (81.9% in poultry, 71.4% in LR) is especially concerning. Fluoroquinolones like ciprofloxacin are essential, first-line agents for treating invasive salmonellosis in humans. Resistance in animal reservoirs directly jeopardizes the efficacy of these drugs in human medicine, leading to longer, more severe illnesses and increased mortality[19]. This finding is consistent with recent Pakistani studies; for instance, research on raw meat in Lahore markets found 84.2% of *Salmonella* isolates resistant to ciprofloxacin[21]. Furthermore, the high prevalence of MDR—70% in poultry and 58.8% in LR—mirrors trends identified in a national meta-analysis, which reported MDR in 7 out of 10 studies on *Salmonella* from Pakistani food animals[22].

Table 12: Key Salmonella findings in context.

Parameter	**Finding from This Passive Surveillance**	**Supporting Evidence from Regional Literature**	**Reference**
Primary Sample Source (Poultry)	Liver (64.2%)	Indicates systemic infection (e.g., fowl typhoid), a severe	Castro-Vergas et al., (2020)[19]

[19] Castro-Vargas, R. E., Herrera-Sánchez, M. P., Rodríguez-Hernández, R., & Rondón-Barragán, I. S. (2020). Antibiotic resistance in Salmonella spp. isolated from poultry: A global overview. Veterinary world, 13(10), 2070–2084. https://doi.org/10.14202/vetworld.2020.2070-2084

[20] Qiu, Y., Ferreira, J. P., Ullah, R. W., Flanagan, P., Zaheer, M. U., Tahir, M. F., Alam, J., Hoet, A. E., Song, J., & Akram, M. (2024). Assessment of the Implementation of Pakistan's National Action Plan on Antimicrobial Resistance in the Agriculture and Food Sectors. Antibiotics, 13(3), 206. https://doi.org/10.3390/antibiotics13030206

[21] Fatima, A., Saleem, M., Nawaz, S. et al. Prevalence and antibiotics resistance status of Salmonella in raw meat consumed in various areas of Lahore, Pakistan. Sci Rep 13, 22205 (2023). https://doi.org/10.1038/s41598-023-49487-2

[22] Arshed, M. J., Umair, M., Talib, U., Tahir, M. F., Abubakar, M., Bahadur, S. U. K., Tahmeena, T., Ullah, R. W., Mohsin, M., Abbas, M. A., Ahsan, Q., Alam, J., & Zaheer, M. U. (2025). Status of antimicrobial resistance in food animals in Pakistan (2016-2020): A systematic review and meta-analysis. Journal of advanced veterinary and animal research, 12(2), 668–679. https://doi.org/10.5455/javar.2025.l930

		clinical presentation leading to lab submission.	
Fluoroquinolone Resistance	Enrofloxacin: 81.9% (Poultry), 71.4% (LR).	A study in Lahore found 84.2% of meat-derived *Salmonella* resistant to ciprofloxacin. Fluoroquinolone misuse in animals drives this critical resistance.	Fatima et al., (2023)[21] Sana et al., (2025)[23]
Multidrug Resistance (MDR)	70% (Poultry), 58.8% (LR).	A national meta-analysis found MDR prevalent in *Salmonella* from Pakistani food animals .	Arshad et al., (2025)[22]
Ampicillin/Amoxicillin Resistance	High prevalence observed (e.g., Ampicillin 93.9% in LR).	The national meta-analysis reports pooled resistance of 78.4% to ampicillin and 53.9% to amoxicillin .	Arshad et al., (2025)[22]
Zoonotic Transmission Risk	Isolation from milk (25.5% in LR) and systemic organs.	Contaminated raw meat and milk are established primary sources of human *Salmonella* infections .	Fatima et al., (2023)[21]

The recovery of *Salmonella* from milk and, in poultry, from eggs and drag swabs, moves the implication beyond animal health into the core of food safety. It provides tangible evidence of product contamination at the point of clinical disease, creating a direct vector for transmitting MDR strains to consumers. This is a significant One Health concern, exacerbated by high rates of raw milk and undercooked meat consumption in the region. The presence of extensively drug-resistant (XDR) *Salmonella* strains, which have been documented in Pakistani retail meat[21], underscores the potential severity of this transmission.

4.3 S. aureus

The sample origin data for *S. aureus* provides crucial context for interpreting its (AMR profile in Pakistan's passive surveillance. In large ruminants, milk was the source for 91.7% of isolates, unequivocally identifying contagious bovine mastitis as the dominant clinical presentation[16]. This aligns with national literature confirming *S. aureus* as the predominant mastitis pathogen in Pakistan[16]. In poultry, however, isolates came from a wider range of sites, including liver (29.6%), trachea (15.1%),

[23] Sana, S. S., Atuahene, D., Nagy, V., Shaikh, A. M., & Knop, R. (2025). The Rising Threat of Antibiotic Resistance in Poultry: Veterinary and One Health Perspectives. Veterinary Sciences, 12(11), 1059. https://doi.org/10.3390/vetsci12111059

and notably, bone marrow (10.1%) and yolk sac (13.2%). This points to infections like septicaemia, respiratory disease, and omphalitis.

The AMR patterns reflect these distinct disease pressures. The very high resistance to tetracyclines (e.g., doxycycline at 93.2% in poultry) and fluoroquinolones is consistent with their common use for treating these conditions. Critically, the detection of methicillin-resistant *S. aureus* (MRSA), indicated by cefoxitin resistance in 40% of poultry and 15.2% of large ruminant isolates tested, represents a serious escalation. A 2025 meta-analysis estimated the overall MRSA prevalence in Pakistan to be 50%[24], and studies of Pakistani food have confirmed the presence of multidrug-resistant and toxigenic *S. aureus* strains[25]. Furthermore, the high MRSA prevalence in chicken meat is a regional concern, with studies in neighboring India finding 27% of *S. aureus* from milk to be MRSA[26].

Table 13: Key S. aureus AMR findings in context

Parameter	Finding from This Passive Surveillance	Supporting Evidence from Regional Literature	Reference
Primary Sample Source (LR)	Milk (91.7%)	Confirms role as primary bovine mastitis pathogen in Pakistan.	Ghumman et al., (2025)[16]
MRSA Detection	Cefoxitin resistance in isolates from poultry (40%) and LR (15.2%).	A national meta-analysis estimates a 50% MRSA prevalence in Pakistan. MRSA is documented in food animals and products.	Ali et al., (2025)[24] Pervaiz et al., (2025)[25]; Deepak et al., (2024)[26]
Multidrug Resistance (MDR)	63.8% (Poultry), 41.4% (LR).	AMR in Staphylococci from Pakistani food animals is documented, with high resistance to penicillin and cefoxitin.	Arshad et al., (2025)[22]
Public Health Link	MRSA in milk, high raw milk consumption.	Establishes a direct foodborne and occupational exposure risk, necessitating One Health interventions.	Ghumman et al., (2025)[16]

[24] Ali, S., Shafiq, M., Aziz, A., Rahman, N. u., & Bakky, M. A. H. (2025). The prevalence of Methicillin-resistant Staphylococcus aureus in Pakistan: A systematic review and meta-analysis. Frontiers in Cellular and Infection Microbiology. Advance online publication. https://doi.org/10.3389/fcimb.2025.1707379

[25] Pervaiz, R., Bano, S., Tunio, S. A., Abro, R., Abbasi, S., & Memon, I. (2025). Antibiotic resistance in foodborne pathogens: Implications for the one health approach in developing nations. Microbial Pathogenesis, *206*, 107737. https://doi.org/10.1016/j.micpath.2025.107737

[26] Deepak, S.J., Kannan, P., Savariraj, W.R. et al. Characterization of Staphylococcus aureus isolated from milk samples for their virulence, biofilm, and antimicrobial resistance. Sci Rep 14, 25635 (2024). https://doi.org/10.1038/s41598-024-75076-y

From a One Health perspective, the isolation of MRSA from mastitic milk is particularly significant. Given Pakistan's high rate of raw milk consumption, this creates a direct transmission route for resistant bacteria to consumers, posing both a food safety and an occupational hazard for dairy workers[15,25]. The data underscores that controlling *S. aureus* and its resistance requires integrated strategies addressing both animal health (through improved mastitis management and antibiotic stewardship) and public health (through food safety measures).

4.4 Policy and practical implications: bridging surveillance data to action

The value of this passive surveillance is realized only if it translates into changed practices. The data provides a direct mandate for action in two key areas:

1. **Informing Veterinary Practice and Stewardship:** The results are a powerful tool for AMS. They provide concrete evidence that certain antibiotic classes are often ineffective for common syndromes like septicemia and mastitis. In addition they provide evidence that HPCIA, CIAs are being used to treat clinical cases . This should catalyze the development of syndrome-specific treatment guidelines promoting culture-guided therapy and the use of narrow-spectrum alternatives where effective. This must also be supported by advice on management practices to reduce the incidence of disease and the need for AMU.

2. **Strengthening the Implementation of National Action Plan:** The findings underscore critical gaps identified in assessments of Pakistan's NAP on AMR. While the NAP established a strategic framework, its implementation in the agriculture sector has been moderate, with many activities remaining in planning or pilot stages and suffering from inadequate regulation and resource constraints. This surveillance data directly supports NAP objectives related to optimizing AMU and strengthening the evidence base. It provides the necessary national evidence to advocate for stricter enforcement of prescription-only policies, enhanced farmer education programs to address knowledge gaps, and greater investment in scalable surveillance and stewardship interventions.

The system for data collection and continuation of passive surveillance is incumbent upon the continued collaboration between MoNFS&R and stakeholders. The government has developed data sharing protocols for laboratories enrolled in passive surveillance in AMR in animal health sector. There are three objectives of system

- To streamline the reporting process of AST results.
- To ensure data consistency, quality, and comparability across reporting sites.
- To enable early detection of AMR trends and inform policy interventions.

Apart from this, national reference laboratories, NVL and NRLPD are working on the harmonization of antibiotic panels to be used for AST in the livestock and poultry sector laboratories in the country.

4.5 Limitations and future direction

The study had a few overarching limitations that must be considered while interpreting the results. Firstly, the pilot study had inherent surveillance system and data integration

restrictions. This study was based on passive AMR surveillance and employed non-standardised AST panels across participating laboratories, with no parallel collection of AMU data. These limitations constrained the interpretation of resistance trends and precluded robust assessment of the relationship between AMR patterns and antimicrobial use practices at the national level, in line with recognised limitations of non-integrated surveillance systems.

Secondly, the data set lacks necessary parameters for accurate prevalence calculation – specifically, the count of samples tested for each specific pathogen. Furthermore, case reporting was incomplete due to selective submission of isolates since some laboratories provided only a subset of positive isolates as per their discretion. The private laboratories, did not have detailed data of animal samples with negative isolation results. This practise results from lack of adequate human resources and data recording systems, which prevents the laboratories from keeping an exhaustive record of all samples. Therefore, the reported results represent frequencies within an incomplete dataset and should not be interpreted as measure of disease burden or true pathogen occurrence.

Thirdly, sampling and laboratory testing methodologies posed a limitation as well. Due to resource and protocol constraints, not all samples were processed for the isolation of each priority bacterial pathogen, potentially resulting in further underestimation of pathogen prevalence and incomplete characterisation of the AMR burden in poultry and livestock populations. The low representation of SR samples, together with the limited number of isolates subjected to AST, further reduced analytical power. In addition, incomplete application of the full AST panel across laboratories may have led to overestimation of resistance to certain antimicrobials, as reflected in the Methods, Results and Discussion sections.

Future steps must build on this foundation:

1. **Standardization:** Implementing a harmonized core AST panel (comprising antimicrobials that are recommended for use in animals) and quantitative data reporting systems.

2. **Integration:** Developing a parallel system for monitoring AMU on farms to directly correlate use with resistance trends.

3. **Expansion:** Moving beyond passive surveillance with periodic active surveys of diseased FPA's focusing on clinical syndromes of highest economic importance like mastitis in LR and collibacillosis in poultry, so as to to map the full resistance landscape.

4. **Regional AMR surveillance:** Regional passive AMR surveillance to monitor for changes in AMR patterns of bacterial pathogens may guide targeted interventions to improve appropriateness of AMU

5 Conclusion

This passive AMR surveillance system, implemented through a representative network of veterinary diagnostic laboratories has successfully demonstrated the feasibility of establishing a functional and nationally representative platform for generating data on pathogen prevalence and AST in Pakistan's livestock and poultry sectors. The findings document a critical reality: pathogens responsible for major clinical diseases exhibit very high levels of resistance to commonly used veterinary antimicrobials. When interpreted appropriately, these results do not reflect population-level resistance prevalence but rather signal widespread therapeutic failure and substantial on-farm selection pressure. The integration of specimen-origin data is pivotal to this interpretation, directly linking resistance patterns to economically and clinically significant disease syndromes such as mastitis and septicemia. Collectively, these findings create an urgent, evidence-based imperative to translate surveillance outputs into actionable AMS guidance and to address key implementation gaps within Pakistan's national AMR containment framework. By strengthening harmonized laboratory methods, sustaining the diagnostic network, and embedding surveillance data into policy and clinical decision-making, this system can achieve its intent by improving animal health outcomes while contributing meaningfully to the One Health response to AMR at the national and global levels to preserve CIAs for future use in humans.

Annex I: Antibiotic breakpoints of the AST panel

Table 14: E. coli breakpoints for the antibiotics tested by the sentinel sites. The breakpoints of antibiotics were primarily taken from CLSI Vet01S (7th Ed) unless stated in the guidline to consult CLSI M100 35th Ed (2025).

Sr. No.	Antibiotics	Antibiotic class	WHO MIA category	Conc. (µg)	S (mm)	I (mm)	R (mm)	Reference
1	Cefoxitin	Cephalosporins	HPCIA	30	≥18	15-17	≤14	M100 35th Ed (2025)
2	Ceftiofur sodium	Cephalosporins	HPCIA	30	≥21	18-20	≤17	VET01S 7th Ed. (2024)
3	Enrofloxacin	Fluoroquinolones	HPCIA	5	≥23	17-22	≤16	VET01S 7th Ed. (2024)
4	Norfloxacin	Fluoroquinolones	HPCIA	10	≥17	13–16	≤12	M100 35th Ed (2025)
5	Fosfomycin	Non B-lactams	HPCIA	200	≥16	13–15	≤12	M100 35th Ed (2025)
6	Gentamycin	Aminoglycosides	CIA	10	≥16	13-15	≤12	VET01S 7th Ed. (2024)
7	Streptomycin	Aminoglycosides	CIA	10	≥15	12–14	≤11	M100 35th Ed (2025)
8	Amoxicillin	Penicillins	HIA	30	≥18	14-17	≤13	M100 35th Ed (2025)
9	Ampicillin	Penicillins	HIA	10	≥17	14-16	≤13	M100 35th Ed (2025)
10	Chloramphenicol	Phenicols	HIA	30	≥18	13-17	≤12	M100 35th Ed (2025)
11	Doxycycline	Tetracyclines	HIA	30	≥14	11--13	≤10	M100 35th Ed (2025)
12	Tetracycline	Tetracyclines	HIA	30	≥15	12-14	≤11	M100 35th Ed (2025)
13	Trimethoprim-Sulfamethoxazole	Folate pathway inhibitors	HIA	25	≥16	11--15	≤10	M100 35th Ed (2025)
14	Nitrofurantoin	Nitrofurans	IA	300	≥ 17	15–16	≤ 14	M100 35th Ed (2025)

Table 15: Salmonella spp. breakpoints for the antibiotics tested by the sentinel sites. The breakpoints of antibiotics were primarily taken from CLSI Vet01S (7th Ed) unless stated in the guidline to consult CLSI M100 35th Ed (2025).

Sr. No.	Antibiotics	Antibiotic class	WHO MIA category	Conc. (µg)	S (mm)	I (mm)	R (mm)	Reference
1	Ceftiofur sodium	Cephalosporins	HPCIA	30	≥ 21	18-20	≤ 17	VET01S 7th Ed. (2024)
2	Enrofloxacin	Fluoroquinolones	HPCIA	5	>23	17-22	<16	VET01S 7th Ed(2024)
3	Gentamicin	Aminoglycosides	CIA	10	>16	13-15	<12	VET01S 7th Ed. (2024)
4	Amoxicillin	Penicillins	HIA	30	≥18	14-17	≤ 13	M100 35th Ed. (2025)
5	Ampicillin	Penicillins	HIA	10	≥ 17	14–16	≤ 13	M100 35th Ed. (2025)
6	Chloramphenicol	Phenicols	HIA	30	≥ 18	13-17	≤ 12	M100 35th Ed. (2025)
7	Doxycycline	Tetracyclines	HIA	30	≥ 14	11--13	≤ 10	M100 35th Ed. (2025)
8	Tetracycline	Tetracyclines	HIA	30	≥ 15	12--14	≤ 11	M100 35th Ed. (2025)
9	Trimethoprim-Sulfamethoxazole	Folate pathway inhibitors	HIA	25	≥ 16	11--15	≤ 10	M100 35th Ed. (2025)

Table 16: Staphylococcus aureus breakpoints for the antibiotics tested by the sentinel sites. The breakpoints of antibiotics were primarily taken from CLSI Vet01S (7th Ed) unless stated in the guidline to consult CLSI M100 35th Ed (2025).

Sr. No.	Antibiotics	Antibiotic class	WHO MIA category	Conc. (µg)	S (mm)	I (mm)	R (mm)	Reference
1	Cefoxitin	Cephalosporins	HPCIA	30	≤ 21	-	≥ 22	CLSI M100 35th Ed. (2025)
2	Enrofloxacin	Fluoroquinolones	HPCIA	5	≤ 16	17-22	≥ 23	VET01S 7th Ed. (2024) (cats)
3	Norfloxacin	Fluoroquinolones	HPCIA	10	≤ 12	13–16	≥ 17	CLSI M100 35th Ed. (2025)
4	Erythromycin	Macrolides	CIA	15	≤ 13	14-22	≥ 23	CLSI M100 35th Ed. (2025)
5	Gentamicin	Aminoglycosides	CIA	10	≤ 12	13–14	≥ 15	CLSI M100 35th Ed. (2025)
6	Chloramphenicol	Amphenicols	HIA	30	≤ 12	13-17	≥ 18	CLSI M100 35th Ed. (2025)

7	Doxycycline	Tetracyclines	HIA	30	≤ 20	21-24	≥ 25	VET01S 7th Ed. (2024)
8	Oxytetracycline	Tetracyclines	HIA	30	≤ 14	15-18	≥ 19	CLSI M100 33rd Ed. (2023)
9	Penicillin	Penicillins	HIA	10	≤ 28	-s	≥ 29	CLSI M100 35th Ed. (2025)
10	Tetracyclin	Tetracyclines	HIA	30	≤ 14	15-18	≥ 19	CLSI M100 35th Ed. (2025)
11	Trimethoprim-Sulfamethoxazole	Folate pathway inhibitors	HIA	25	≤ 10	11--15	≥ 16	CLSI M100 35th Ed. (2025)
12	Nitrofurantoin	Nitrofurans	IA	300	≤ 14	15–16	≥ 17	CLSI M100 35th Ed. (2025)

Annex II: Sample types and antibiotic resistance profile against target bacterial species in diseased FPA

*Table 17: The table details the frequency (f) of each sample type for poultry and its corresponding percentage for E. coli, Salmonella spp. and S. aureus. The **majority** of the samples for all three target pathogens were collected post-mortem.*

Sample Types of Poultry	*E. coli (f)*	*%*	*Salmonella spp. (f)*	*%*	*S. aureus (f)*	*%)*
Blister	1	0.1	0	0	1	0.3
Blood	1	0.1	0	0	0	0
Bone Marrow	0	0	0	0	32	10.1
carcass	6	0.5	0	0	0	0
Ceacum	59	4.7	0	0	0	0
Cloaca	7	0.6	11	2.1	2	0.6
Dead chick	13	1	0	0	20	6.3
Drag Swabs	0	0	29	5.5	2	0.6
Eggs	2	0.2	24	4.6	0	0
Faeces	9	0.7	14	2.7	0	0
Feed samples	1	0.1	1	0.2	2	0.6
Hatchery swabs	1	0.1	0	0	1	0.3
Heart	3	0.2	9	1.7	5	1.6
Hock	0	0	0	0	1	0.3
Intestines	14	1.1	1	0.2	0	0
Joint	0	0	0	0	15	4.7
Kidney	1	0.1	0	0	0	0
Liver	875	69.1	336	64.2	94	29.6
Lungs	100	7.9	20	3.8	18	5.7
Meat sample	1	0.1	0	0	0	0
Oral	8	0.6	13	2.5	14	4.4
Other	5	0.4	3	0.6	8	2.5
Oviduct	2	0.2	0	0	0	0
Proventriculus	2	0.2	10	1.9	1	0.3
Semen	6	0.5	0	0	4	1.3
Spleen	25	2	13	2.5	8	2.5
Swab	1	0.1	1	0.2	0	0
Tissue	3	0.2	3	0.6	0	0
Trachea	59	4.7	32	6.1	48	15.1
Yolk	20	1.6	3	0.6	0	0
Yolk Sac	42	3.3	0	0	42	13.2
Total	1267	100	523	100	318	100

Table 18:The table details the frequency (f) of each sample type from LR and their corresponding percentage for E. coli, Salmonella spp. and S. aureus. The majority of the samples for all three target pathogens were collected post-mortem

Sample Types of LR	***E. coli* (*f*)**	***%***	***Salmonella spp. (f)***	***%***	***S. aureus (f)***	***%)***
Blood	5	3.3	0	0	4	2.5
Ceacum	0	0	0	0	0	0
Faeces	7	4.6	5	9.8	0	0
Heart	0	0	2	3.9	0	0
Intestine	2	1.3	2	3.9	0	0
Kidney	1	0.7	3	5.9	0	0
Liver	3	2	5	9.8	2	1.3
Lung	2	1.3	3	5.9	2	1.3
Meat	2	1.3	1	2	0	0
Milk	125	81.7	13	25.5	144	91.7
Oral	0	0	0	0	0	0
Other	2	1.3	11	21.6	3	1.9
Rectal	0	0	0	0	0	0
Serum	1	0.7	2	3.9	0	0
Spleen	0	0	2	3.9	0	0
Tissues	0	0	0	0	0	0
Tongue	1	0.7	1	2	1	0.6
Trachea	1	0.7	1	2	1	0.6
Urine	1	0.7	0	0	0	0
Total	153		51		157	

Table 19: The table details the frequency (f) of each sample type from SR and their corresponding percentage for E. coli, Salmonella spp. and S. aureus. The majority of the samples for all three target pathogens were collected post-mortem

Sample Types of SR	***E. coli* (*f*)**	***%***	***Salmonella spp. (f)***	***%***	***S. aureus (f)***	***%)***
Blood	2	5.7	0	0.0	0	0.0
Ceacum	2	5.7	0	0.0	0	0.0
Faeces	5	14.3	0	0.0	0	0.0
Heart	0	0	0	0.0	0	0.0
Intestine	0	0	2	9.5	0	0.0
Kidney	5	14.3	4	19.0	0	0.0
Liver	5	14.3	5	23.8	1	7.1
Lung	7	20	6	28.6	1	7.1
Meat	0	0	0	0.0	0	0.0
Milk	2	5.7	0	0.0	6	42.9
Oral	0	0	0	0.0	5	35.7
Other	1	2.9	0	0.0	0	0.0

Rectal	0	0	1	4.8	0	0.0
Serum	0	0	0	0.0	0	0.0
Spleen	5	14.3	3	14.3	1	7.1
Tissues	1	2.9	0	0.0	0	0.0
Tongue	0	0	0	0.0	0	0.0
Trachea	0	0	0	0.0	0	0.0
Urine	0	0	0	0.0	0	0.0
Total	35		21		14	

Table 20: Antibiotic resistance profile for E. coli in different animal groups (large ruminants, small ruminants and poultry) showing resistance rates (R%) with 95% confidence intervals. R=Count of Resistant isolates, I= Count of Intermediate isolates.

Animal Group	WHO Category	Antibiotic Class	Antibiotic	R+I	Total isolates	R%	95% CI
Large ruminants	HPCIA	Cephalosporin	Cefoxitin	2	2	100.0	34.2 - 100
		Fluoroquinolones	Enrofloxacin	42	47	89.4	77.4 - 95.4
		Fluoroquinolones	Norfloxacin	23	38	60.5	44.7 - 74.4
		Fosfomycin	Fosfomycin	2	27	7.4	2.1 - 23.4
	CIA	Aminoglycosides	Gentamicin	43	151	28.5	21.9 - 36.1
		Aminoglycosides	Streptomycin	39	46	84.8	71.8 - 92.4
	HIA	Amphenicol	Chloramphenicol	33	112	29.5	21.8 - 38.5
		Folate Pathway Inhibitors	Trimethoprim-Sulfamethoxazole	59	101	58.4	48.7 - 67.5
		Penicillin	Amoxicillin	33	33	100.0	89.6 - 100
		Penicillin	Ampicillin	116	124	93.5	87.8 - 96.7
		Tetracycline	Doxycycline	32	32	100.0	89.3 - 100
		Tetracycline	Tetracycline	57	101	56.4	46.7 - 65.7
	IA	Nitrofuran	Nitrofurantoin	12	12	100.0	75.8 - 100
Small ruminants	HPCIA	Fluoroquinolones	Enrofloxacin	9	9	100.0	70.1 - 100
		Fluoroquinolones	Norfloxacin	6	24	25.0	12 - 44.9
		Fosfomycin	Fosfomycin	0	16	0.0	0 - 19.4
	CIA	Aminoglycosides	Gentamicin	7	35	20.0	10 - 35.9
		Aminoglycosides	Streptomycin	4	5	80.0	37.6 - 96.4
	HIA	Amphenicol	Chloramphenicol	6	11	54.5	28 - 78.7
		Folate Pathway Inhibitors	Trimethoprim-Sulfamethoxazole	6	10	60.0	31.3 - 83.2
		Penicillin	Amoxicillin	7	7	100.0	64.6 - 100
		Penicillin	Ampicillin	28	31	90.3	75.1 - 96.7
		Tetracycline	Doxycycline	6	6	100.0	61 - 100
		Tetracycline	Tetracycline	6	11	54.5	28 - 78.7
	IA	Nitrofuran	Nitrofurantoin	1	1	100.0	20.7 - 100
Poultry	HPCIA	Cephalosporins	Cefoxitin	19	37	51.4	35.9 - 66.6
		Cephalosporins	Ceftiofur	202	557	36.3	32.4 - 40.3
		Fluoroquinolones	Enrofloxacin	942	1048	89.9	87.9 - 91.6
		Fluoroquinolones	Norfloxacin	607	811	74.8	71.7 - 77.7

		Phosphonic Acids	Fosfomycin	190	592	32.1	28.5 - 36
	CIA	Aminoglycosides	Gentamicin	551	1063	51.8	48.8 - 54.8
		Aminoglycosides	Streptomycin	454	476	95.4	93.1 - 96.9
	HIA	Penicillins	Amoxicillin	372	426	87.3	83.8 - 90.2
		Penicillins	Ampicillin	626	640	97.8	96.4 - 98.7
		Amphenicols	Chloramphenicol	266	334	79.6	75 - 83.6
		Tetracyclines	Doxycycline	742	1117	66.4	63.6 - 69.1
		Tetracyclines	Tetracycline	182	202	90.1	85.2 - 93.5
		Folate Pathway Inhibitors	Trimethoprim-Sulfamethoxazole	604	673	89.7	87.2 - 91.8
	IA	Nitrofurans	Nitrofurantoin	27	42	64.3	49.2 - 77

Table 21: Antibiotic resistance profile for Salmonella spp. in different animal groups (large ruminants, small ruminants and poultry) showing resistance rates (R%) with 95% confidence intervals. R=Count of Resistant isolates, I= Count of Intermediate isolates.

Animal Group	WHO Category	Antibiotic Class	Antibiotic	R+I	Total isolates	R%	95% CI
Large ruminants	HPCIA	Fluoroquinolones	Enrofloxacin	25	35	71.4	54.9 - 83.7
	CIA	Aminoglycosides	Gentamicin	19	51	37.3	25.3 - 51
	HIA	Penicillin	Amoxicillin	22	23	95.7	79 - 99.2
		Penicillin	Ampicillin	46	49	93.9	83.5 - 97.9
		Amphenicol	Chloramphenicol	15	16	93.8	71.7 - 98.9
		Tetracycline	Doxycycline	28	46	60.9	46.5 - 73.6
		Tetracycline	Tetracycline	18	20	90.0	69.9 - 97.2
		Folate Pathway Inhibitors	Trimethoprim-Sulfamethoxazole	16	16	100.0	80.6 - 100
Small ruminants	HPCIA	Fluoroquinolones	Enrofloxacin	10	20	50.0	29.9 - 70.1
	CIA	Aminoglycosides	Gentamicin	6	20	30.0	14.5 - 51.9
	HIA	Penicillin	Amoxicillin	10	10	100.0	72.2 - 100
		Penicillin	Ampicillin	15	18	83.3	60.8 - 94.2
		Amphenicol	Chloramphenicol	2	3	66.7	20.8 - 93.9
		Tetracycline	Doxycycline	7	19	36.8	19.1 - 59
		Tetracycline	Tetracycline	2	2	100.0	34.2 - 100
		Folate Pathway Inhibitors	Trimethoprim-Sulfamethoxazole	2	2	100.0	34.2 - 100
Poultry	HPCIA	Cephalosporins	Ceftiofur	43	147	29.3	22.5 - 37.1
	HPCIA	Fluoroquinolones	Enrofloxacin	371	453	81.9	78.1 - 85.2
	CIA	Aminoglycosides	Gentamicin	223	471	47.3	42.9 - 51.9
	HIA	Penicillins	Amoxicillin	208	243	85.6	80.6 - 89.5
	HIA	Penicillins	Ampicillin	335	411	81.5	77.5 - 85
	HIA	Amphenicols	Chloramphenicol	137	190	72.1	65.3 - 78
	HIA	Tetracyclines	Doxycycline	377	495	76.2	72.2 - 79.7
	HIA	Tetracyclines	Tetracycline	92	123	74.8	66.5 - 81.6
	HIA	Folate Pathway Inhibitors	Trimethoprim-Sulfamethoxazole	105	183	57.4	50.1 - 64.3

Table 22: Antibiotic resistance profile for Staphylococcus aureus in different animal groups (large ruminants, small ruminants and poultry) showing resistance rates (R%) with 95% confidence intervals. R=Count of Resistant isolates, I= Count of Intermediate isolates.

Animal Group	WHO Category	Antibiotic Class	Antibiotic	R+I	Total isolates	R%	95% CI
Large Ruminants	HPCIA	Cephalosporins	Cefoxitin	5	33	15.2	6.7 - 30.9
		Fluoroquinolones	Enrofloxacin	30	83	36.1	26.6 - 46.9
		Fluoroquinolones	Norfloxacin	34	37	91.9	78.7 - 97.2
	CIA	Macrolides	Erythromycin	39	57	68.4	55.5 - 79
		Aminoglycosides	Gentamicin	31	92	33.7	24.9 - 43.8
	HIA	Amphenicols	Chloramphenicol	26	85	30.6	21.8 - 41
		Tetracyclines	Doxycycline	38	46	82.6	69.3 - 90.9
		Tetracyclines	Oxytetracycline	25	32	78.1	61.2 - 89
		Penicillins	Penicillin	77	137	56.2	47.8 - 64.2
		Tetracyclines	Tetracycline	55	86	64.0	53.4 - 73.3
		Folate Pathway Inhibitors	Trimethoprim-Sulfamethoxazole	28	77	36.4	26.5 - 47.5
Small Ruminants	HPCIA	Cephalosporins	Cefoxitin	1	2	50.0	9.5 - 90.5
		Fluoroquinolones	Enrofloxacin	4	10	40.0	16.8 - 68.7
		Fluoroquinolones	Norfloxacin	3	3	100.0	43.9 - 100
	CIA	Aminoglycosides	Gentamicin	4	6	66.7	30 - 90.3
		Macrolides	Erythromycin	2	3	66.7	20.8 - 93.9
	HIA	Amphenicols	Chloramphenicol	0	3	0.0	0 - 56.1
		Tetracyclines	Doxycycline	1	2	50.0	9.5 - 90.5
		Tetracyclines	Oxytetracycline	5	11	45.5	21.3 - 72
		Penicillins	Penicillin	11	13	84.6	57.8 - 95.7
		Tetracyclines	Tetracycline	1	3	33.3	6.1 - 79.2
		Folate Pathway Inhibitors	Trimethoprim-Sulfamethoxazole	1	3	33.3	6.1 - 79.2
Poultry	HPCIA	Cephalosporins	Cefoxitin	8	20	40	21.9 - 61.3
	HPCIA	Fluoroquinolones	Enrofloxacin	221	250	88.4	83.8 - 91.8
	HPCIA	Fluoroquinolones	Norfloxacin	142	193	73.6	66.9 - 79.3

	CIA	Macrolides	Erythromycin	47	52	90.4	79.4 - 95.8
	CIA	Aminoglycosides	Gentamicin	80	190	42.1	35.3 - 49.2
	HIA	Amphenicols	Chloramphenicol	90	120	75	66.6 - 81.9
	HIA	Tetracyclines	Doxycycline	207	222	93.2	89.2 - 95.9
	HIA	Tetracyclines	Oxytetracycline	172	186	92.5	87.8 - 95.5
	HIA	Penicillins	Penicillin	91	109	83.5	75.4 - 89.3
	HIA	Tetracyclines	Tetracycline	37	40	92.5	80.1 - 97.4
	HIA	Folate Pathway Inhibitors	Trimethoprim-Sulfamethoxazole	118	156	75.6	68.3 - 81.7
	IA	Nitrofurans	Nitrofurantoin	4	11	36.4	15.2 - 64.6

Table 23: Multiclass resistance profile of bacterial isolates in different animal groups. The table details the frequency of each isolates showing resistance to the antibiotic classes, along with their respective percentage (resistant isolates in the class/total isolates) m total counts of E. coli (LR = 153, SR = 35, poultry = 1267), Salmonella spp. (LR =51, SR = 21, poultry = 523) and S. aureus (LR = 157, SR = 14, poultry = 318).

Bacteria	Antibiotic class	Large ruminants (Frequency (Percentage))	Small ruminants (Frequency (Percentage))	Poultry (Frequency (Percentage))
E. coli	0 classes	18 (11.8%)	1 (2.9%)	16 (1.3%)
	1 class	41 (26.8%)	21 (60%)	79 (6.2%)
	2 classes	23 (15%)	3 (8.6%)	174 (13.7%)
	3 classes	20 (13.1%)	4 (11.4%)	296 (23.4%)
	4 classes	18 (11.8%)	0 (0.0%)	316 (24.9%)
	5 classes	21 (13.7%)	5 (14.3%)	165 (13%)
	6 classes	0 (0.0%)	0 (0.0%)	165 (13%)
	7 classes	12 (7.8%)	1 (2.9%)	46 (3.6%)
	8 classes	-	-	10 (0.8%)
Salmonella spp.	1 class	10 (19.6%)	9 (42.9%)	7 (1.3%)
	2 classes	11 (21.6%)	7 (33.3%)	42 (8%)
	3 classes	14 (27.5%)	2 (9.5%)	108 (20.7%)
	4 classes	3 (5.9%)	1 (4.8%)	143 (27.3%)
	5 classes	11 (21.6%)	2 (9.5%)	151 (28.9%)
	6 classes	2 (3.9%)	0 (0.0%)	66 (12.6%)
S. aureus	0 classes	35 (22.3%)	1 (7.1%)	3 (0.9%)
	1 class	35 (22.3%)	6 (42.9%)	31 (9.7%)
	2 classes	22 (14%)	2 (14.3%)	81 (25.5%)
	3 classes	27 (17.2%)	1 (7.1%)	89 (28%)
	4 classes	19 (12.1%)	4 (28.6%)	69 (21.7%)
	5 classes	14 (8.9%)	0 (0.0%)	40 (12.6%)
	6 classes	4 (2.5%)	0 (0.0%)	3 (0.9%)
	7 classes	1 (0.6%)	0 (0.0%)	2 (0.6%)

Table 24: Distribution of multidrug resistance (MDR) patterns among E. coli isolates from large ruminants (n = 153), small ruminants (n = 35) and poultry (n = 1267) detailing the specific combination of antibiotic classes, antibiotic combination

*phenotype, acronym for the combination, the number of classes resisted, and the frequency of each pattern. *For poultry, an exhaustive list of antibiotic combinations (count = 314) was identified, therefore only one combination per class with the highest frequency is mentioned in the table.*

Animal Group	No. of resistant classes	Antibiotic Classes	Antibiotic combination phenotype	Acronym	Frequency
Large ruminants	3	Penicillin, Fluoroquinolone, Aminoglycoside	Amoxicillin-Enrofloxacin-Gentamicin-Norfloxacin	AMX-ENR-GEN-NOR	8
	3	Penicillin, Aminoglycoside, Folate pathway inhibitor	Ampicillin-Streptomycin-Trimethoprim–Sulfamethoxazole	AMP-STR-SXT	4
	3	Penicillin, Tetracycline, Folate pathway inhibitor	Ampicillin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMP-TCY-SXT	2
	4	Penicillin, Aminoglycoside, Tetracycline, Folate pathway inhibitor	Ampicillin-Streptomycin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMP-STR-TCY-SXT	12
	4	Penicillin, Aminoglycoside, Tetracycline, Folate pathway inhibitor	Ampicillin-Gentamicin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMP-GEN-TCY-SXT	3
	4	Penicillin, Aminoglycoside, Tetracycline, Folate pathway inhibitor	Ampicillin-Gentamicin-Streptomycin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMP-GEN-STR-TCY-SXT	2
	5	Penicillin, Phenicol, Tetracycline, Fluoroquinolone, Folate pathway inhibitor	Amoxicillin-Ampicillin-Chloramphenicol-Doxycycline-Enrofloxacin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMX-AMP-CHL-DOX-ENR-TCY-SXT	20
	7	Penicillin, Phenicol, Tetracycline, Fluoroquinolone, Aminoglycoside, Nitrofuran, Folate pathway inhibitor	Ampicillin-Chloramphenicol-Doxycycline-Enrofloxacin-Gentamicin-Nitrofurantoin-Norfloxacin-Streptomycin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMP-CHL-DOX-ENR-GEN-NIT-NOR-STR-TCY-SXT	7
	7	Penicillin, Phenicol, Tetracycline, Fluoroquinolone, Aminoglycoside, Nitrofuran, Folate pathway inhibitor	Ampicillin-Chloramphenicol-Doxycycline-Enrofloxacin-Gentamicin-Nitrofurantoin-Streptomycin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMP-CHL-DOX-ENR-GEN-NIT-STR-TCY-SXT	3
	7	Penicillin, Cephalosporin (2nd gen), Phenicol, Tetracycline, Fluoroquinolone, Nitrofuran, Folate pathway inhibitor	Amoxicillin-Ampicillin-Cefoxitin-Chloramphenicol-Doxycycline-Enrofloxacin-Nitrofurantoin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMX-AMP-FOX-CHL-DOX-ENR-NIT-TCY-SXT	2
Small ruminants	3	Penicillin, Fluoroquinolone, Aminoglycoside	Amoxicillin-Enrofloxacin-Gentamicin-Norfloxacin	AMX-ENR-GEN-NOR	2
	3	Penicillin, Aminoglycoside, Fluoroquinolone	Ampicillin-Gentamicin-Norfloxacin	AMP-GEN-NOR	2
	5	Penicillin, Phenicol, Tetracycline, Fluoroquinolone, Folate pathway inhibitor	Amoxicillin-Ampicillin-Chloramphenicol-Doxycycline-Enrofloxacin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMX-AMP-CHL-DOX-ENR-TCY-SXT	5
	7	Penicillin, Phenicol, Tetracycline, Fluoroquinolone, Aminoglycoside, Nitrofuran, Folate pathway inhibitor	Ampicillin-Chloramphenicol-Doxycycline-Enrofloxacin-Gentamicin-Nitrofurantoin-Norfloxacin-Streptomycin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMP-CHL-DOX-ENR-GEN-NIT-NOR-STR-TCY-SXT	1
Poultry*	3	Tetracycline, Fluoroquinolone, Folate pathway inhibitor	Doxycycline-Enrofloxacin-Norfloxacin-Trimethoprim-Sulfamethoxazole	DOX-ENR-NOR-SXT	45
	4	Tetracycline, Fluoroquinolone, Aminoglycoside, Folate pathway inhibitor	Doxycycline-Enrofloxacin-Gentamicin-Norfloxacin-Trimethoprim-Sulfamethoxazole	DOX-ENR-GEN-NOR-SXT	108
	5	Penicillin, Phenicol, Tetracycline, Fluoroquinolone, Aminoglycoside, Folate pathway inhibitor	Amoxicillin-Ampicillin-Chloramphenicol-Doxycycline-Enrofloxacin-Streptomycin-Trimethoprim-Sulfamethoxazole	AMX-AMP-CHL-DOX-ENR-STR-SXT	15

	6	Penicillin, Phenicol, Tetracycline, Fluoroquinolone, Aminoglycoside, Folate pathway inhibitor	Ampicillin-Chloramphenicol-Doxycycline-Enrofloxacin-Streptomycin-Tetracycline-Trimethoprim-Sulfamethoxazole	AMP-CHL-DOX-ENR-STR-TCY-SXT	28
	7	Penicillin, Phenicol, Tetracycline, Fluoroquinolone, Aminoglycoside, Folate pathway inhibitor	Amoxicillin-Ampicillin-Chloramphenicol-Doxycycline-Enrofloxacin-Gentamicin-Norfloxacin-Streptomycin-Trimethoprim-Sulfamethoxazole	AMX-AMP-CHL-DOX-ENR-GEN-NOR-STR-SXT	6
	8	Penicillin, Phenicol, Tetracycline, Fluoroquinolone, Aminoglycoside, Nitrofuran, Folate pathway inhibitor	Ampicillin-Chloramphenicol-Doxycycline-Enrofloxacin-Gentamicin-Nitrofurantoin-Norfloxacin-Streptomycin-Tetracycline-Trimethoprim-Sulfamethoxazole	AMP-CHL-DOX-ENR-GEN-NIT-NOR-STR-TCY-SXT	14

*Table 25: Distribution of multidrug resistance (MDR) patterns among Salmonella isolates from large ruminants (n = 51), small ruminants (n = 21) and poultry (n = 523) detailing the specific combination of antibiotic classes, antibiotic combination phenotype, acronym for the combination, the number of classes resisted, and the frequency of each pattern. *For poultry, an exhaustive list of antibiotic combinations (count = 117) was identified, therefore only one combination per class with the highest frequency is mentioned in the table.*

Animal Group	**No. of resistant classes**	**Antibiotic Classes**	**Antibiotic combination phenotype**	**Acronym**	**Frequency**
Large ruminants	3	Penicillin, Tetracyclines, Fluoroquinolones	Ampicillin-Doxycycline-Enrofloxacin	AMP-DOX-ENR	2
	3	Penicillin, Fluoroquinolones, Aminoglycosides	Ampicillin-Enrofloxacin-Gentamicin	AMP-ENR-GEN	2
	3	Penicillin, Aminoglycosides, Tetracyclines	Ampicillin-Gentamicin-Tetracycline	AMP-GEN-TCY	2
	2	Penicillin, Tetracyclines	Amoxicillin-Ampicillin-Doxycycline	AMX-AMP-DOX	1
	3	Penicillin, Tetracyclines, Fluoroquinolones	Amoxicillin-Ampicillin-Doxycycline-Enrofloxacin	AMX-AMP-DOX-ENR	5
	3	Penicillin, Fluoroquinolones, Aminoglycosides	Amoxicillin-Ampicillin-Enrofloxacin-Gentamicin	AMX-AMP-ENR-GEN	3
	4	Penicillin, Phenicols, Tetracyclines, Folate Pathway Inhibitors	Ampicillin-Chloramphenicol-Doxycycline-Tetracycline-Trimethoprim–Sulfamethoxazole	AMP-CHL-DOX-TCY-SXT	2
	4	Penicillin, Tetracyclines, Fluoroquinolones, Aminoglycosides	Amoxicillin-Ampicillin-Doxycycline-Enrofloxacin-Gentamicin	AMX-AMP-DOX-ENR-GEN	1
	5	Penicillin, Phenicols, Tetracyclines, Aminoglycosides, Folate Pathway Inhibitors	Ampicillin-Chloramphenicol-Doxycycline-Gentamicin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMP-CHL-DOX-GEN-TCY-SXT	5
	5	Penicillin, Phenicols, Tetracyclines, Fluoroquinolones, Folate Pathway Inhibitors	Amoxicillin-Ampicillin-Chloramphenicol-Doxycycline-Enrofloxacin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMX-AMP-CHL-DOX-ENR-TCY-SXT	6

	6	Penicillin, Phenicols, Tetracyclines, Fluoroquinolones, Aminoglycosides, Folate Pathway Inhibitors	Amoxicillin-Ampicillin-Chloramphenicol-Doxycycline-Enrofloxacin-Gentamicin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMX-AMP-CHL-DOX-ENR-GEN-TCY-SXT	2
Small ruminants	3	Penicillin, Tetracyclines, Fluoroquinolones	Ampicillin-Doxycycline-Enrofloxacin	AMP-DOX-ENR	1
	3	Penicillin, Tetracyclines, Fluoroquinolones	Amoxicillin-Ampicillin-Doxycycline-Enrofloxacin	AMX-AMP-DOX-ENR	1
	4	Penicillin, Tetracyclines, Fluoroquinolones, Aminoglycosides	Amoxicillin-Ampicillin-Doxycycline-Enrofloxacin-Gentamicin	AMX-AMP-DOX-ENR-GEN	1
	5	Penicillin, Phenicols, Tetracyclines, Fluoroquinolones, Folate Pathway Inhibitors	Amoxicillin-Ampicillin-Chloramphenicol-Doxycycline-Enrofloxacin-Tetracycline-Trimethoprim–Sulfamethoxazole	AMX-AMP-CHL-DOX-ENR-TCY-SXT	2
Poultry*	3	Penicillin, Tetracycline, Fluoroquinolone	Amoxicillin + Ampicillin + Doxycycline + Enrofloxacin	AMX-AMP-DOX-ENR	31
	4	Penicillin, Tetracycline, Fluoroquinolone, Aminoglycoside	Ampicillin + Doxycycline + Enrofloxacin + Gentamicin	AMP-DOX-ENR-GEN	44
	5	Penicillin, Phenicol, Tetracycline, Fluoroquinolone, Aminoglycoside	Amoxicillin + Ampicillin + Chloramphenicol + Doxycycline + Enrofloxacin + Gentamicin	AMX-AMP-CHL-DOX-ENR-GEN	14
	6	Penicillin, Phenicol, Tetracycline, Fluoroquinolone, Folate pathway inhibitor	Amoxicillin + Ampicillin + Chloramphenicol + Doxycycline + Enrofloxacin + Tetracycline + Trimethoprim-Sulfamethoxazole	AMX-AMP-CHL-DOX-ENR-TCY-SXT	10

*Table 26: Distribution of multidrug resistance (MDR) patterns among S. aureus isolates from large ruminants (n = 157), small ruminants (n = 14) and poultry (n = 318) detailing the specific combination of antibiotic classes, antibiotic combination phenotype, acronym for the combination, the number of classes resisted, and the frequency of each pattern. *For poultry, an exhaustive list of antibiotic combinations (count = 65) was identified, therefore only one combination per class with the highest frequency is mentioned in the table.*

Animal Group	**No. of resistant classes**	**Antibiotic Classes**	**Antibiotic combination phenotype**	**Acronym**	**Frequency**
Large ruminants	3	Tetracyclines, Macrolides, Beta-lactams	Doxycycline-Erythromycin-Penicillin	DOX-ERY-PEN	7
	3	Phenicols, Tetracyclines, Folate Pathway Inhibitors	Chloramphenicol-Tetracycline-Trimethoprim–Sulfamethoxazole	CHL-TCY-SXT	5
	3	Phenicols, Fluoroquinolones, Tetracyclines	Chloramphenicol-Enrofloxacin-Tetracycline	CHL-ENR-TCY	2
	3	Cephalosporins, Macrolides, Beta-lactams	Cefoxitin-Erythromycin-Penicillin	FOX-ERY-PEN	1
	3	Phenicols, Macrolides, Aminoglycosides	Chloramphenicol-Erythromycin-Gentamicin	CHL-ERY-GEN	1
	3	Fluoroquinolones, Beta-lactams, Tetracyclines	Enrofloxacin-Norfloxacin-Penicillin-Tetracycline	ENR-NOR-PEN-TCY	3
	3	Tetracyclines, Macrolides, Beta-lactams	Doxycycline-Erythromycin-Penicillin-Tetracycline	DOX-ERY-PEN-TCY	2
	3	Fluoroquinolones, Tetracyclines, Beta-lactams	Enrofloxacin-Norfloxacin-Oxytetracycline-Penicillin	ENR-NOR-OXY-PEN	2

	3	Fluoroquinolones, Aminoglycosides, Tetracyclines	Enrofloxacin-Gentamicin-Norfloxacin-Oxytetracycline-Tetracycline	ENR-GEN-NOR-OXY-TCY	3
	3	Fluoroquinolones, Tetracyclines, Beta-lactams	Enrofloxacin-Norfloxacin-Oxytetracycline-Penicillin-Tetracycline	ENR-NOR-OXY-PEN-TCY	1
	4	Tetracyclines, Macrolides, Beta-lactams, Folate Pathway Inhibitors	Doxycycline-Erythromycin-Penicillin-Trimethoprim–Sulfamethoxazole	DOX-ERY-PEN-SXT	2
	4	Cephalosporins, Tetracyclines, Macrolides, Beta-lactams	Cefoxitin-Doxycycline-Erythromycin-Penicillin	FOX-DOX-ERY-PEN	1
	4	Phenicols, Fluoroquinolones, Beta-lactams, Tetracyclines	Chloramphenicol-Enrofloxacin-Penicillin-Tetracycline	CHL-ENR-PEN-TCY	1
	4	Fluoroquinolones, Aminoglycosides, Tetracyclines, Beta-lactams	Enrofloxacin-Gentamicin-Oxytetracycline-Penicillin	ENR-GEN-OXY-PEN	1
	4	Fluoroquinolones, Aminoglycosides, Tetracyclines, Beta-lactams	Enrofloxacin-Gentamicin-Norfloxacin-Oxytetracycline-Penicillin	ENR-GEN-NOR-OXY-PEN	7
	4	Cephalosporins, Tetracyclines, Macrolides, Beta-lactams	Cefoxitin-Doxycycline-Erythromycin-Penicillin-Tetracycline	FOX-DOX-ERY-PEN-TCY	1
	4	Tetracyclines, Macrolides, Fluoroquinolones, Folate Pathway Inhibitors	Doxycycline-Erythromycin-Norfloxacin-Tetracycline-Trimethoprim–Sulfamethoxazole	DOX-ERY-NOR-TCY-SXT	1
	4	Fluoroquinolones, Aminoglycosides, Tetracyclines, Beta-lactams	Enrofloxacin-Gentamicin-Norfloxacin-Oxytetracycline-Penicillin-Tetracycline	ENR-GEN-NOR-OXY-PEN-TCY	5
	5	Phenicols, Tetracyclines, Macrolides, Beta-lactams, Folate Pathway Inhibitors	Chloramphenicol-Doxycycline-Erythromycin-Penicillin-Trimethoprim–Sulfamethoxazole	CHL-DOX-ERY-PEN-SXT	1
	5	Tetracyclines, Macrolides, Fluoroquinolones, Beta-lactams, Folate Pathway Inhibitors	Doxycycline-Erythromycin-Norfloxacin-Penicillin-Trimethoprim–Sulfamethoxazole	DOX-ERY-NOR-PEN-SXT	1
	5	Tetracyclines, Macrolides, Fluoroquinolones, Beta-lactams, Folate Pathway Inhibitors	Doxycycline-Erythromycin-Norfloxacin-Penicillin-Tetracycline-Trimethoprim–Sulfamethoxazole	DOX-ERY-NOR-PEN-TCY-SXT	4
	5	Tetracyclines, Macrolides, Aminoglycosides, Fluoroquinolones, Folate Pathway Inhibitors	Doxycycline-Erythromycin-Gentamicin-Norfloxacin-Tetracycline-Trimethoprim–Sulfamethoxazole	DOX-ERY-GEN-NOR-TCY-SXT	3
	5	Phenicols, Tetracyclines, Macrolides, Aminoglycosides, Folate Pathway Inhibitors	Chloramphenicol-Doxycycline-Erythromycin-Gentamicin-Tetracycline-Trimethoprim–Sulfamethoxazole	CHL-DOX-ERY-GEN-TCY-SXT	2
	5	Cephalosporins, Tetracyclines, Macrolides, Aminoglycosides, Beta-lactams	Cefoxitin-Doxycycline-Erythromycin-Gentamicin-Penicillin-Tetracycline	FOX-DOX-ERY-GEN-PEN-TCY	1

	5	Phenicols, Tetracyclines, Macrolides, Beta-lactams, Folate Pathway Inhibitors	Chloramphenicol-Doxycycline-Erythromycin-Penicillin-Tetracycline-Trimethoprim–Sulfamethoxazole	CHL-DOX-ERY-PEN-TCY-SXT	1
	5	Tetracyclines, Macrolides, Aminoglycosides, Beta-lactams, Folate Pathway Inhibitors	Doxycycline-Erythromycin-Gentamicin-Penicillin-Tetracycline-Trimethoprim–Sulfamethoxazole	DOX-ERY-GEN-PEN-TCY-SXT	1
	6	Tetracyclines, Macrolides, Aminoglycosides, Fluoroquinolones, Beta-lactams, Folate Pathway Inhibitors	Doxycycline-Erythromycin-Gentamicin-Norfloxacin-Penicillin-Trimethoprim–Sulfamethoxazole	DOX-ERY-GEN-NOR-PEN-SXT	1
	6	Phenicols, Tetracyclines, Macrolides, Aminoglycosides, Fluoroquinolones, Folate Pathway Inhibitors	Chloramphenicol-Doxycycline-Erythromycin-Gentamicin-Norfloxacin-Tetracycline-Trimethoprim–Sulfamethoxazole	CHL-DOX-ERY-GEN-NOR-TCY-SXT	**2**
	6	Cephalosporins, Tetracyclines, Macrolides, Aminoglycosides, Beta-lactams, Folate Pathway Inhibitors	Cefoxitin-Doxycycline-Erythromycin-Gentamicin-Penicillin-Tetracycline-Trimethoprim–Sulfamethoxazole	FOX-DOX-ERY-GEN-PEN-TCY-SXT	**1**
	7	Phenicols, Tetracyclines, Macrolides, Aminoglycosides, Fluoroquinolones, Beta-lactams, Folate Pathway Inhibitors	Chloramphenicol-Doxycycline-Erythromycin-Gentamicin-Norfloxacin-Penicillin-Tetracycline-Trimethoprim–Sulfamethoxazole	CHL-DOX-ERY-GEN-NOR-PEN-TCY-SXT	1
Small ruminants	3	Aminoglycosides, Beta-lactams, Folate Pathway Inhibitors	Gentamicin-Penicillin-Trimethoprim–Sulfamethoxazole	GEN-PEN-SXT	1
	4	Fluoroquinolones, Aminoglycosides, Tetracyclines, Beta-lactams	Enrofloxacin-Gentamicin-Norfloxacin-Oxytetracycline-Penicillin	ENR-GEN-NOR-OXY-PEN	3
	4	Cephalosporins, Tetracyclines, Macrolides, Beta-lactams	Cefoxitin-Doxycycline-Erythromycin-Penicillin-Tetracycline	FOX-DOX-ERY-PEN-TCY	1
Poultry*	3	Fluoroquinolone, Tetracycline, Penicillin	Enrofloxacin-Oxytetracycline-Penicillin	ENR-OXY-PEN	33
	4	Phenicol, Fluoroquinolone, Tetracycline, Folate pathway inhibitor	Chloramphenicol-Enrofloxacin-Tetracycline-Trimethoprim-Sulfamethoxazole	CHL-ENR-TCY-SXT	6
	5	Phenicol, Tetracycline, Fluoroquinolone, Folate pathway inhibitor	Chloramphenicol-Doxycycline-Enrofloxacin-Norfloxacin-Oxytetracycline-Trimethoprim-Sulfamethoxazole	CHL-DOX-ENR-NOR-OXY-SXT	24
	6	Phenicol, Tetracycline, Fluoroquinolone, Aminoglycoside, Folate pathway inhibitor	Chloramphenicol-Doxycycline-Enrofloxacin-Gentamicin-Norfloxacin-Oxytetracycline-Trimethoprim-Sulfamethoxazole	CHL-DOX-ENR-GEN-NOR-OXY-SXT	19
	7	Phenicol, Tetracycline, Macrolide, Aminoglycoside, Penicillin, Folate pathway inhibitor	Chloramphenicol-Doxycycline-Erythromycin-Gentamicin-Norfloxacin-Penicillin-Tetracycline-Trimethoprim-Sulfamethoxazole	CHL-DOX-ERY-GEN-NOR-PEN-TCY-SXT	2

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