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Study of Antibiotic Resistance of *Escherichia coli* strains isolated from livestock in the province of Guelma

Presented by **M^iss KIRAT Hassina**

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Dedication

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Hassna

Table of contents

Methods

Results

Abstract

Antibiotic resistance is a global health concern, threatening both human and veterinary medicine, with livestock acting as a major reservoir for the dissemination and persistence of resistant bacteria particularly third-generation cephalosporin-resistant (3GC-R) and colistin-resistant (CL-R) Enterobacterales. This thesis investigated the dissemination of 3GC-R and CL-R *Escherichia coli* among poultry, bovine and ovine farms in Guelma province, northeastern Algeria. A longitudinal surveillance was further performed in order to assess the persistence of CL-R isolates in poultry farms over production cycles. A total of 919 animal and environmental samples were collected from 38 farms in Guelma. Samples were screened for 3GC-R and CL-R *E. coli* in selective media. The screened isolates were identified then subjected to susceptibility tests, including the disc diffusion test, the determination of colistin minimum inhibitory concentration, and the phenotypic detection of extended spectrum β-lactamase and carbapenemase enzymes. Afterwards, standard PCR was performed to the 3GC-R isolates to investigate the presence of antibiotic resistance genes and integrons, whereas the CL-R were submitted to whole genome sequencing. Overall, 112 isolates were collected from 21 farms, including 58 3GC-R and 54 CL-R *E. coli* strains. The 3GC-R isolates were detected in poultry, bovine and ovine farms, while CL-R strains were only found in poultry farms. Phenotypic tests revealed that the majority of the isolates were multidrug resistant and displayed high resistance rates against β-lactams, tetracyclines, fluoroquinolones and sulfonamides. The molecular analysis identified critical resistance genes including *mcr-1*, $bla_{\text{CTX-M}}$, bla_{NDM}, $bla_{\text{OXA-181}}$, bla_{CMY} and bla_{DHA}. The MLST analysis identified 28 different Sequence Types (STs), mainly ST162 and ST93, and provided evidence on the clonal dissemination and the potential persistence of several STs within the farms. These findings underscore the major role of livestock as a reservoir for resistant bacteria and their genes and emphasize the urgent need for targeted strategies within a "One Health" framework to mitigate the spread of these isolates and preserve the effectiveness of critical antibiotic agents.

Key words: Antibiotic-resistant *E. coli*, Colistin, Third-generation cephalosporins, ESBL, Carbapenemase, AmpC, MCR, Livestock.

Résumé

L'antibiorésistance est un problème de santé publique mondial, menaçant la médecine humaine et vétérinaire, le bétail agissant comme un réservoir majeur pour la dissémination et la persistance des bactéries résistantes, en particulier les entérobactéries résistantes aux céphalosporines de troisième génération (3GC-R) et à la colistine (CL-R). Cette thèse a porté sur la dissémination d'*Escherichia coli* 3GC-R et CL-R parmi les fermes avicoles, bovines et ovines dans la wilaya de Guelma, nord-est de l'Algérie. Une surveillance longitudinale a été ensuite réalisée afin d'évaluer la persistance des isolats CL-R dans les élevages de volailles au cours des cycles de production. Un total de 919 échantillons animaux et environnementaux ont été collectés dans 38 fermes à Guelma. Les échantillons ont été criblés pour la recherche d'*E. coli* 3GC-R et CL-R dans des milieux sélectifs et ensuite identifiés puis soumis à des tests de sensibilité, y compris le test de diffusion de disque, la détermination de la concentration minimale inhibitrice de la colistine, et la détection phénotypique de la production de β-lactamases à spectre étendu et de carbapénémases. Ensuite, une PCR standard a été réalisée sur les isolats 3GC-R pour rechercher la présence de gènes de résistance aux antibiotiques et d'intégrons, tandis que les CL-R ont été soumis à un séquençage du génome complet. Au total, 112 isolats ont été collectés dans 21 fermes, y compris 58 souches d'*E. coli* 3GC-R et 54 souches d'*E. coli* CL-R. Les isolats 3GC-R ont été détectés dans les élevages aviaires, bovins et ovins, tandis que les souches CL-R n'ont été trouvées que chez les volailles. Les tests phénotypiques ont révélé que la majorité des isolats étaient multirésistants et présentaient des taux de résistance élevés contre les β-lactamines, les tétracyclines, les fluoroquinolones et les sulfonamides. L'analyse moléculaire a identifié des gènes de résistance critiques, y compris *mcr-1*, $bla_{\text{CTX-M}}$, bla_{NDM}, $bla_{\text{OXA-181}}$, bla_{CMY} et bla_{DHA}. L'analyse MLST a identifié 28 types de séquence différents (STs), principalement ST162 et ST93, et a mis en évidence la dissémination clonale et la persistance potentielle de plusieurs STs au sein des fermes. Ces résultats indiquent le rôle majeur des animaux d'élevage en tant que réservoir de bactéries résistantes et de leurs gènes et mettent l'accent sur l'urgence d'adopter des stratégies ciblées dans le cadre d'une approche «One Health» pour atténuer la propagation de ces souches et préserver l'efficacité des agents antimicrobiens critiques.

Mots clés : *E. coli* résistant aux antibiotiques, Colistine, Céphalosporines de troisième génération, BLSE, Carbapénémase, AmpC, MCR, Animaux d'élevage.

ملخص

مقاومة المضادات الحيوية هي قضية صحية عالمية تهدد كل من الطب البشري والبيطرية، حيث تلعب المواشي دورًا حيويًا في انتشار وبقاء البكتيريا المقاومة وتعمل كخزان رئيسي لسلالات خطيرة، لا سيما السلالات البكتيريا المعوية المقاومة للجيل الثالث من السيفالوسبورينات (3GC-R) والمقاومة للكولستين (CL-R). تحققت هذه الأطروحة من انتشار *Escherichia coli* المقاومة للجيل الثالث من السيفالوسبورينات والمقاومة للكوليستين في مزارع الدواجن والأبقار والأغنام في مدينة قالمة، شمال شرق الجزائر. تم ايضا إجراء مراقبة طولية لتقييم استمرار عزلات CL-R في مزارع الدواجن على مدى دورات الإنتاج. تم جمع 919 عينة من الحيوانات والبيئة من 38 مزرعة في قالمة. تم فحص العينات بحثًا عن بكتيريا *E. coli* المقاومة للجيل الثالث من السيفالوسبورينات والمقاومة للكوليستين في الأوساط الانتقائية. ثم تم فحصها واخضاعها لاختبارات الحساسية، بما في ذلك اختبار انتشار الأقراص، وتحديد الحد الأدنى من التركيز المثبط للكولستين، والكشف الظاهري عن إنتاج انزيمات بيتالاكتاماز واسعة الطيف والكاربينيماز. بعد ذلك، تم إجراء PCR لعزلات GC-R3 للتحقق من وجود جينات مقاومة المضادات الحيوية والإنتجرونات، بينما تم إخضاع عزلات CL-R لتسلسل الجينوم الكامل. بشكل عام، تم جمع 112 عزلة من 21 مزرعة، بما في ذلك 58 سلالة من سلالات *E. coli* المقاومة للجيل الثالث من السيفالوسبورينات و54 سلالة من سلالات *E. coli* المقاومة للكوليستين. تم اكتشاف عزلات (GC-R3) في مزارع الدواجن والأبقار والأغنام، بينما تم العثور على سلالات (CL-R) فقط في مزارع الدواجن. كشفت الاختبارات الظاهرية أن غالبية العزلات كانت متعددة المقاومة للأدوية وأظهرت معدلات مقاومة عالية ضد البيتا لاكتامات، والتتراسيكلينات، والفلوروكينولونات، والسلفونواميدات. حدد التحليل الجزيئي جينات مقاومة حرجة بما في ذلك*mcr-1* ، $bla_{\text{CTX-M}}$، bla_{NDM}، $bla_{\text{OXA-181}}$، bla_{CMY} و bla_{DHA}. حدد تحليل MSLT 28 نوعًا مختلفًا من الأنماط التسلسلية(STs) ، خاصة ST162وST93 ، وقدم أدلة على الانتشار النسلي والإمكانية المحتملة لبقاء عدة STs داخل المزارع. تؤكد هذه النتائج الدور الكبير للماشية كخزان للبكتيريا المقاومة وجيناتها وتبرز الحاجة الملحة لاستراتيجيات مستهدفة ضمن إطار " One Health" للتخفيف من انتشار هذه العزلات والحفاظ على كفاءة المضادات الحيوية الحرجة.

الكلمات المفتاحية: *E. coli* المقاومة للمضادات الحيوية، كوليستين، السيفالوسبورينات من الجيل الثالث، إنزيمات ESBL ، إنزيمات كربابينيماز، إنزيمات AmpC ، إنزيمات MCR ، المواشي.

List of tables

List of figures

Abbreviations

3GC	Third-Generation Cephalosporins
3GC-R	Third-Generation Cephalosporin Resistant
AK	Amikacin
AM	Ampicillin
AMC	Amoxicillin-Clavulanic Acid
AMR	Antimicrobial resistance
ATM	Aztreonam
Ca	Calcium
Ca-MHB	Cation Adjusted Mueller Hinton Broth
CAZ	Ceftazidime
CDC	Centers Of Disease Control and Prevention
CDT	Combination Disc Test
CIP	Ciprofloxacin
CIPRAS	Canadian Integrated Program for Antimicrobial Resistance Surveillance
CL	Colistin
CLSI	Clinical And Laboratory Standards Institute
CN	Gentamicin
CTX	Cefotaxime
DDST	Double Disc Synergy Test
DNA	Deoxyribonucleic Acid
DO	Doxycycline
ECDC	European Center for Disease Control and Prevention
EDTA	Ethylenediaminetetraacetic Acid
EFSA	European Food Safety Authority

EMA	European Medicines Agency
ESBL	Extended Spectrum β-Lactamase
ETP	Ertapenem
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FEP	Cefepime
FOX	Cefoxitin
HGT	Horizontal Gene Transfer
IMP	Imipenem
LPS	Lipopolysaccharide
MALDI-TOF ms	Matrix-Assisted Laser Desorption Ionization–Time Of Flight Mass Spectrometry
MCR	Mobile Colistin Resistance
MDR	Multidrug Resistance
Mg	Magnesium
MH	Mueller Hinton
MHB	Mueller Hinton Broth
MIC	Minimal Inhibitory Concentration
MLST	Multilocus Sequence Typing
NA	Nalidixic Acid
OFX	Ofloxacin
PBP	Penicillin-Binding Protein
PCR	Polymerase Chain Reaction
PDR	Pandrug Resistance
PEtn	Phosphoethanolamine

RNA	Ribonucleic Acid
SNP	Single Nucleotide Polymorphism
ST	Sequence Type
SXT	Trimethoprim-Sulfamethoxazole
TE	Tetracycline
WGS	Whole Genome Sequencing
WHO	World Health Organization
WOAH	World Organization for Animal Health
XDR	Extensively Drug Resistance

Introduction

Since their first discovery in the early twentieth century, antibiotics have revolutionized therapeutic practices in both human and veterinary medicine allowing the treatment and prevention of previously fatal bacterial infections and significantly improving life expectancy worldwide (Hutchings et al., 2019). These molecules of natural or synthetic origin act by killing or inhibiting target bacteria through multiple mechanisms, while maintaining low or negligible toxicity towards the host cell (Brooks & Jawetz, 2013).

Beyond their clinical use in human medicine, antibiotics have played a crucial role in the veterinary sector, where they have been widely used for therapeutic and non-therapeutic purposes ensuring disease treatment and control, promoting animal growth and improving their welfare, which has significantly enhanced the economic productivity and sustainability of the agricultural industry and made these agents indispensable in most farming practices (Halawa et al., 2024). Recent reports has approximated that over two-thirds of the antibiotics sold worldwide are used in animal husbandry and thus exceeding their overall consumption in the human sector (Mallioris et al., 2023; Wee et al., 2020).

This widespread use, and often misuse of antibiotics has led to the selection and dissemination of resistant bacteria in all ecological niches including humans, animals, environment and food products (Holmes et al., 2016). The phenomenon of antibiotic resistance represents a major global concern for the public health since it threatens the efficacy of critical antibiotics and contributes to the increasing of morbidity and mortality rates worldwide (O′Neill, 2016; WHO, 2022a).

The bacterial resistance against antibiotics occurs naturally or under a selective pressure applied by the extensive use of these agents and enables bacteria to evade the action of antibiotics through multiple mechanisms, most of which can be transmitted between strains vertically or via horizontal gene transfer (HGT) and further accelerating the propagation of resistant bacteria among different hosts (Sengupta et al., 2013; WHO, 2022b). This serious health threat is of a particularly pronounced in the case of critically important antibiotics, such as cephalosporins, carbapenems and colistin, which are the last resort therapeutic options and where the loss the efficiency compromises the ability to treat life-threatening infections (WHO, 2024b).

β-lactams have long been the most commonly used class of antibiotics in both human and veterinary medicine (Kim et al., 2023). Among these, cephalosporines, mainly third-

generations and higher, and carbapenems were classified by the World Health Organization (WHO) as critically important agents given their pivotal role in combating serious infections (WHO, 2024b). However, the widespread use of β-lactams has led to the development and the spread of resistant bacteria, undermining the effectiveness of these crucial agents. The most critical resistance mechanism to β-lactams is the production of β-lactamase enzymes, particularly extended spectrum β-lactamases (ESBLs), AmpC enzymes, and carbapenemases, capable of hydrolyzing nearly all β-lactam antibiotics (Kim et al., 2023). These enzymes are of great concern and have been widely and increasingly reported in different bacterial hosts and across multiple niches including humans, animals and the environment. Most importantly, ESBLs, AmpCs and carbapenemase enzymes are often encoded on plasmid-mediated genes enabling their transmission between species and further complicating their mitigation (Brooks & Jawetz, 2013; Madec et al., 2017).

Parallel to β-lactams, colistin has served as a last-line antibiotic for the treatment of multi-drug resistant (MDR) Gram-negative bacteria, particularly carbapenem-resistant strains (Touati & Mairi, 2021). Although its clinical use in humans was limited owing to its toxicity, colistin was extensively applied in the veterinary sector, especially in animal husbandry, not only for therapeutic purposes, but also for prophylaxis, metaphylaxis and growth promotion (EFSA & ECDC, 2025). This broad application has induced the selection of colistin resistance in many bacterial species and their spread across different niches, mainly farm animals (Khine et al., 2022). Colistin resistance has long been attributed to chromosomal mutations, until Liu et al., 2016 have discovered a new plasmid-encoded gene named *mcr-1*, that confers colistin resistance in multiple Gram-negative species, primarily *Escherichia coli* (Mondal et al., 2024). Since then, nine *mcr* homologs were identified (*mcr-2* to *mcr-10*), with more than twenty-two variants (Liu et al., 2024). These transferable genes have marked a turning point in the understanding of colistin resistance mechanisms, since their horizontal spread between bacterial hosts and across ecological reservoirs has complicated the management of this resistance, turning it into a major public health concern.

Antibiotic resistance in food-producing animals represents a critical challenge for public health and a global economic burden. In fact, the emergence and dissemination of resistant bacteria among livestock undermine the efficacy of antibiotic treatment and increase the incidence of infectious diseases, threatening both animal and human health, which in turn has major outcomes on the global economic productivity (O'Neill, 2015; Pell et al., 2025). The

wide consumption of antibiotics in livestock has promoted the selection and the persistence of resistant bacteria in the animal gut, which acts as a dynamic reservoir for the carriage and the amplification of resistant strains as well as the exchange of resistance genes between commensal and pathogenic bacteria via HGT (Hu et al., 2016; Li et al., 2020). Following their fecal excretion from the animal gut, resistant bacteria can spread through multiple routes, including direct contact, contaminated water or food products, manures, and other vectors, enabling their transmission between human, animal and environmental reservoirs (Founou et al., 2016; Tang et al., 2017).

Among the gastrointestinal microbiome, *E. coli* plays a central role both as a commensal and pathogenic bacterium and is also recognized as a major reservoir for resistance genes (Poirel et al., 2018). Therefore, this species has gained considerable interest in antibiotic resistance investigations and has been commonly employed as an indicator organism, owing to its widespread and ubiquitous presence among the animal and human gut microbiota, its genetic adaptability and ease of isolation and analysis and, most notably, its broad capacity to acquire and transfer resistance determinants (Davies & Wales, 2019; Poirel et al., 2018). The surveillance of antibiotic-resistant *E. coli* in livestock provides valuable insights into the potential animal reservoirs from which resistant bacteria and their genetic determinants can be transmitted to human populations, and is therefore of great importance to public health (EFSA & ECDC, 2025).

The magnitude of the antibiotic resistance problem in livestock extends beyond the veterinary sector and represents a serious threat to human and environmental health as well. In fact, the animal production represents a major reservoir for the amplification and the dissemination of resistant bacteria to human and environmental niches through multiple pathways (Holmes et al., 2016). Besides, the majority of antibiotics used in livestock are likewise administered in humans, and thus the misuse of these agents in the animal sector threatens their effectiveness in both human and veterinary medicine (WHO, 2017b). The interconnectedness of human, animal and environmental health has been broadly advocated by several international organizations, including the WHO, under the concept of “One Health” which emerges as a collaborative multidisciplinary framework aiming to address global health challenges like antibiotic resistance within an integrated and cross-sectoral strategy (WHO, 2017b). Accordingly, several national and international action plans have been implemented in

line with the global One Health strategy, intended to monitor and mitigate the antibiotic resistance within a coordinated and effective framework (WHO, 2015).

The central problematic of the present thesis is the lack of understanding of the mechanisms and the dynamics driving the emergence, the spread and the persistence of resistant bacteria and their genetic determinants among livestock and their environment. To address this gap, this study investigated the dissemination of third-generation cephalosporin-resistant (3GC-R) and colistin-resistant (CL-R) *E. coli* in poultry, bovine and ovine farms in Guelma province, northeast of Algeria. The primary objectives of the study were to assess the fecal carriage of 3GC-R and CL-R *E. coli* in poultry, cattle and sheep, to characterize their phenotypic and molecular resistance profiles, and to evaluate the impact of various factors on their prevalence. A longitudinal investigation was further conducted in order to investigate the long-term persistence of CL-R isolates in poultry farms across successive production cycles.

This thesis is structured into four main chapters reflecting its aims and its overall approach. It begins with a literature review exploring antibiotic background, the concept of antibiotic resistance, its mechanisms and its implication in the veterinary sector, followed by a review of the resistance mechanisms against critically important antibiotics, particularly β-lactams and colistin, and their spread among livestock, and lastly, an overview of the global strategy against antibiotic resistance within the "One Health" framework. The second chapter of this thesis describes the methodological approach conducted, including the samples collection, the bacterial isolation and identification as well as the phenotypic and molecular characterization of the resistance mechanisms. The subsequent chapter represents the findings obtained in this study, supported by tables and graphical illustrations. These results are discussed in the fourth chapter in the light of previous studies, emphasizing their relevance and broader implication. Finally, the thesis ended with a general conclusion, summarizing the main results of this study and their outcomes and providing general perspectives and recommendations for future research.

Literature Review

I. Antibiotic resistance from a veterinary perspective

I.1. Overview of antibiotics

I.1.1. Definition

The term "antibiotic" originates from the Greek roots "*anti* = against" and "*bios* = life", which defines a natural or synthetic molecule that kills targeted bacteria or inhibits their growth at low concentrations, without serious toxicity to the host (Dyary et al., 2023; Kar, 2007; Walsh, 2003). A key concept of antibiotic action is their "selective toxicity" towards the target bacteria without being harmful to the hosting organism (Brooks & Jawetz, 2013).

Reportedly, the utilization of antibiotic-producing microorganisms for infection treatment dates back more than 2000 years, where several ancient civilizations applied moldy bread to infected wounds (Dyary et al., 2023). However, the golden era of antibiotics started with their accidental discovery by Alexander Fleming in 1928 and culminated in the 1950s, when they were widely introduced in clinical practices and significantly revolutionized the treatment of infections (Hutchings et al., 2019).

I.1.2. Classification

Antibiotics can be classified based on various properties, mainly their origin, their antibacterial action, their spectrum of activity, their target site on the bacterial cell as well as their chemical structure.

a. Origin-based classification

- **Natural antibiotics**

A natural metabolite produced by one microorganism (bacteria or fungi), in a particular environment, to either kill neighboring organisms or inhibit their activity (e.g., polymyxin produced by *Paenbacillus polymyxa* and penicillin from *Penicillium* species) (Alagarsamy, 2010).

- **Synthetic antibiotics**

Synthetic antibiotics are wholly man-made agents, developed in the lab through chemical processes to exert similar effects to natural antibiotics (e.g., quinolones and sulfonamides) (Haddad et al., 2024).

- **Semi-synthetic antibiotics**

Semi-synthetic antibiotics are modified compounds derived from natural antibiotics with improved properties (e.g., amoxicillin derived from penicillin and cephalexin modified from cephalosporin) (Walsh, 2003).

b. Action-based classification

- **Bactericidal antibiotics**

Bactericidal antibiotics directly kill target bacteria by attacking its vital components or preventing essential processes (e.g., penicillin and aminoglycosides) (Walsh, 2003).

- **Bacteriostatic antibiotics**

The bacteriostatic action of antibiotics consists of the inhibition of the growth and the reproduction of target bacteria, facilitating their elimination by the immune system (e.g., chloramphenicol and tetracyclines) (Walsh, 2003).

c. Spectrum-based classification

- **Broad spectrum**

Antibiotics that act against a wide range of bacteria, including Gram-positive and Gram-negative species (e.g., fluoroquinolones and tetracyclines) (Dyary et al., 2023).

- **Narrow spectrum**

A narrow-spectrum antibiotic can target a limited range of bacterial species, often targeting either Gram-negative or Gram-positive groups (e.g., colistin and aztreonam) (Dyary et al., 2023).

d. Target-based classification

- **Inhibition of cell wall synthesis**

This class of antibiotics directly targets the bacterial cell wall, inducing its destruction or the inhibition of its production (e.g., penicillins and cephalosporins) (Brooks & Jawetz, 2013).

- **Inhibition of cell membrane function**

Several antibiotics disrupt the cytoplasmic membrane integrity, leading to the leakage of cell content and bacterial death (e.g., polymyxins) (Alagarsamy, 2010).

- **Inhibition of protein synthesis**

Many antibiotics interfere with the protein metabolism through different mechanisms, mainly by affecting the ribosome functions (e.g., aminoglycosides and tetracyclines) (Alagarsamy, 2010).

- **Inhibition of nucleic acid synthesis**

These antibiotics act by inhibiting either bacterial DNA or RNA synthesis (e.g., quinolones and rifampicin) (Brooks & Jawetz, 2013) (Figure 1).

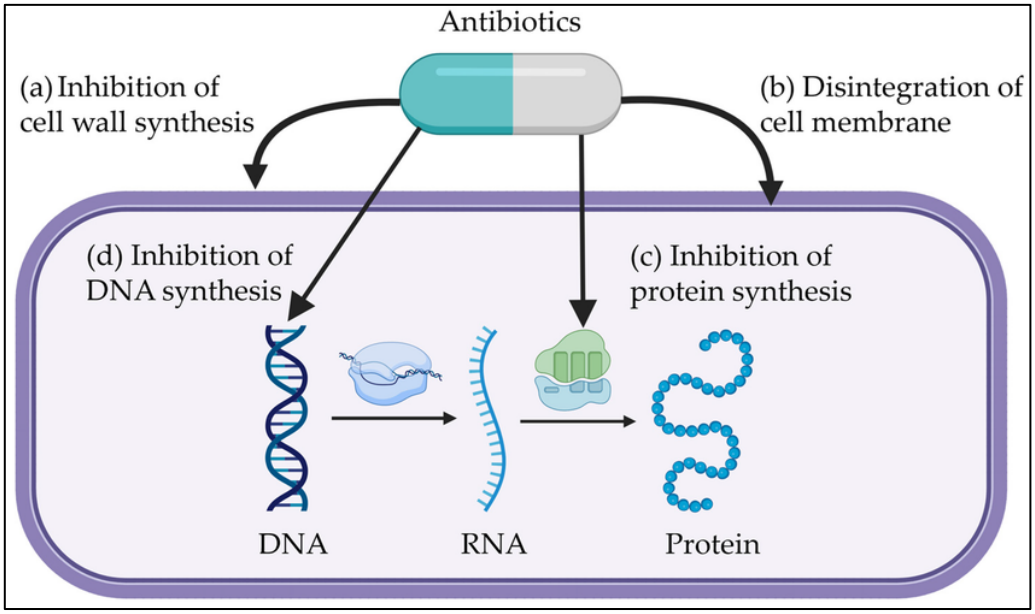

Figure 1: Antibiotic target and mechanism of action. (Sung et al., 2025)

e. Chemical structure

Antibiotics are classified into families and classes based on their shared chemical core structure, named the core scaffold. This core is the fundamental backbone of the drug that remains conserved across derivatives and defines its biological function (Table 1) (Fischbach & Walsh, 2009).

Table 1: Chemical structure (core scaffold) of the main antibiotic families

Antibiotic family	Chemical properties	Examples
β-lactams	4-membered β-lactam ring	Amoxicillin, cefotaxime, ertapenem, aztreonam
Tetracyclines	Four linearly fused rings with keto-enol groups	Tetracycline, doxycycline
Quinolones/ Fluoroquinolones	Quinoline bicyclic nucleus	Ciprofloxacin, nalidixic acid
Macrolides	Macrocyclic lactone ring	Erythromycin, azithromycin
Aminoglycosides	2-deoxystreptamine nucleus	Gentamicin, Amikacin, streptomycin
Polypeptides	Cyclic or linear peptide chains	Colistin, polymyxin B
Sulfonamides	p-aminobenzenesulfonamide core	Sulfamethoxazole-Trimethoprim
Glycopeptides	Heptapeptide core with aromatic residues	Vancomycin, dalbavancin
Lincosamides	Amino-octose sugar linked to proline derivative	Lincomycin, clindamycin
Rifamycins	Ansamycin core	Rifampicin, Rifabutin
Nitroimidazoles	Imidazole ring with -NO_2 group	Metronidazole, Tinidazole
Oxazolidinones	Oxazolidinone heterocyclic ring	Linezolid, Tedizolid

Streptogramins	Polyunstructural cyclic lactone core	Quinupristine-Dalfoprostine
Other scaffolds	Nitrobenzene core	Chloramphenicol
	Steroidal core	Fusidic acid
	cyclic lipopeptide	Daptomycin

I.2. Background on antibiotic resistance

I.2.1. Definition and emergence

Antibiotic resistance is defined by the World Health Organization (WHO) as the ability of bacteria to resist the effect of antibiotic agents that would normally kill them or inhibit their growth (WHO, 2022b).

Antibiotic resistance is an ancient and natural phenomenon predating the antibiotic era, on which bacteria have traditionally relied to adapt and survive in natural environments where antibiotic substances are produced by competing antagonists (Dyary et al., 2023; Pereira et al., 2021).

However, the evolution and spread of this resistance have been significantly intensified since the discovery of antibiotics and their extensive application in human and animal sectors (Holmes et al., 2016). In fact, the selective pressure applied by the widespread use of antibiotics has promoted the rapid emergence and dissemination of resistant bacteria as well as their resistance determinants (Sengupta et al., 2013).

A major outcome arising from the selection of antibiotic resistance in pathogenic bacteria is their evolution into multidrug-resistant (MDR), extensively drug-resistant (XDR), or even pan-drug-resistant (PDR) strains, which limits the treatment options for infectious diseases (Aslam et al., 2018; Davies & Wales, 2019).

I.2.2. Mechanisms

Bacterial resistance to antibiotics arises through different mechanisms that may act separately or concurrently in order to provide high resistance against several antibiotic agents at once, by either (i) reducing membrane permeability to limit antibiotic entry, (ii) expelling the antibiotic via efflux pumps, (iii) altering the antibiotic target to prevent its binding, (iv)

modifying the metabolic pathway to bypass the reaction targeted by the antibiotic, or (v) inactivating or destroying the antibiotic with specific enzymes (Figure 2) (Brooks & Jawetz, 2013; Halawa et al., 2024; Laws et al., 2019).

Moreover, antibiotic resistance can be intrinsic or acquired. Intrinsic resistance exists naturally in a bacterial group and consists of structural or metabolic properties that confer innate resistance to antibiotics (Laws et al., 2019). In contrast, acquired resistance is not consistently present in all strains of a bacterial species; rather, it evolves from spontaneous mutations and spreads via vertical transmission or horizontal gene transfer (HGT), which further accelerates the spread of resistance across bacterial populations (Laws et al., 2019; Pereira et al., 2021).

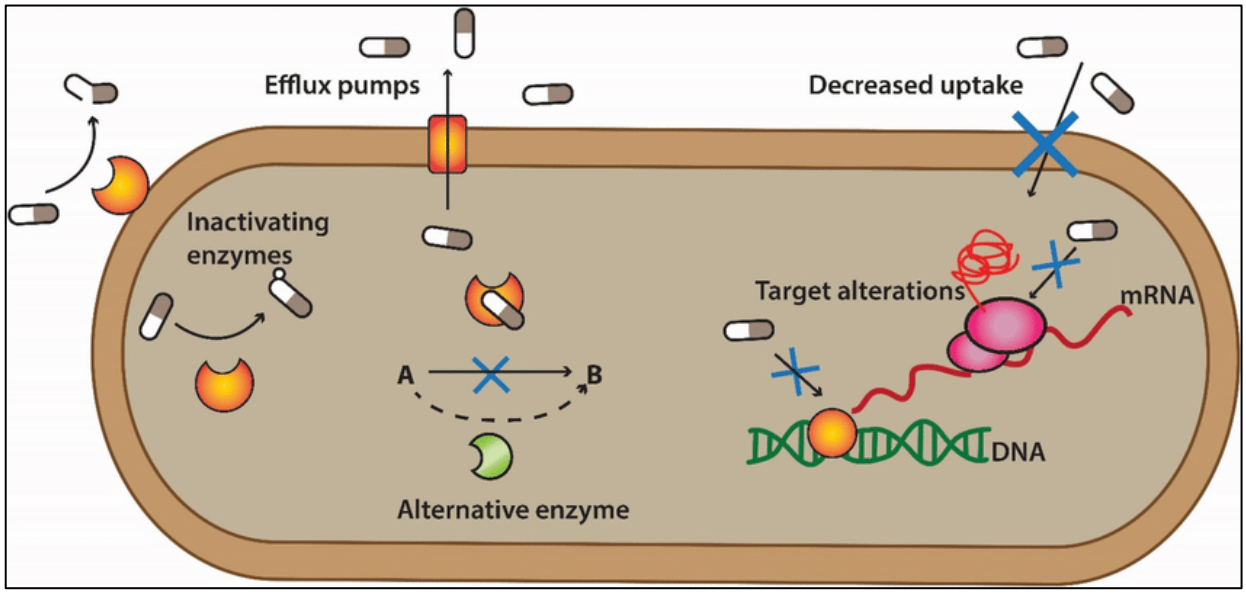

Figure 2: Mechanisms of bacterial resistance against antibiotics. (Mutuku et al., 2022)

I.3. Antibiotic resistance in animals

I.3.1. The role of antibiotic use in the emergence of resistance in livestock

The global consumption of antibiotics in the veterinary sector has dramatically increased over the last decades, exceeding the overall quantity used in the human sector (Wee et al., 2020). It was approximated that 73% of the antibiotics sold worldwide are consumed in animal husbandry, with projections of further escalation if no interventions are implemented (Mallioris et al., 2023).

The widespread use of antibiotics in food-producing animals is one of the key drivers of the global antibiotic resistance concern. Antibiotics are often administered in livestock for three main purposes: (i) therapeutic use for the treatment of infections and non-therapeutic use, either (ii) as prophylactic agents for the prevention of infections or as (iii) growth promoters (Halawa et al., 2024). The non-therapeutic application of antibiotics consists of their administration over extended periods at sub-therapeutic doses, which significantly increases the selection of resistant strains (Founou et al., 2016). These practices have been banned in multiple high-income countries while they remain common in low- and middle-income countries (Hickman et al., 2021).

The massive use of antibiotics in food-producing animals is amplified by the global intensification of livestock production and the poor biosecurity conditions that induce the vulnerability of animals to infections (Founou et al., 2016; Holmes et al., 2016; Pereira et al., 2021). Additionally, the lack of awareness among farmers and the negligence in veterinary oversight further increase the inadequate application of antibiotics (Founou et al., 2016; Holmes et al., 2016).

I.3.2. Dissemination pathways of antibiotic resistance from livestock

a. Direct transmission

Direct human-animal contact contributes to the immediate spread of resistant bacteria within the two hosts. This transmission is more frequent in farmers, veterinarians and individuals in proximity with livestock and their biological material (e.g., feces, milk, blood…) (Founou et al., 2016).

b. Foodborne transmission

Animal-derived food products constitute a significant vehicle for the dissemination of resistant bacteria along the food chain. The risk of acquisition of such bacteria is further increased by the insufficient cooking or inadequate manipulation (Founou et al., 2016).

c. Environmental transmission

The environment serves as a critical reservoir for the persistence and transmission of antibiotic resistant bacteria and their genes. Water environments, including seawater, rivers,

drinking water, irrigation water and wastewater, play a crucial role in the mobilization of resistant strains mainly from farmlands and hospitals and their dispersal across different environments (Baquero et al., 2008; Marti et al., 2014). Likewise, soil ecosystems contribute to the storage and the emission of resistant bacteria. The introduction of such strains in soils, mainly through human and animal wastes, manures as well as urban and hospital sewages, facilitates their access to the food chain via the contamination of vegetables and fruits and their emission in aquatic environments (Gentile et al., 2024; Zhang et al., 2019).

d. Wildlife and insects

Wild animals and insects constitute important vectors facilitating the spread of antibiotic resistance across farms, urban habitats, and natural environments (Davies & Wales, 2019). On the other hand, migratory birds play a crucial role in the long-distance propagation of resistant bacteria and their genetic determinants (Davies & Wales, 2019).

I.3.3. Global impact of antibiotic resistance in livestock

a. Implications for public health

The spread of antibiotic resistance among food-producing animals has adverse outcomes on both human and animal health. In fact, the proliferation of resistant bacteria among animals compromises the treatment of zoonotic infections allowing their spread into the human population. Besides, the decreased antibiotic activity threatens fundamental medical procedures, such as chemotherapy, surgery, transplantation and intensive care, which are heavily relying on the effectiveness of infections control (Ackers et al., 2020; Jamrozik & Selgelid, 2020). On the other hand, antibiotic resistance has a direct impact on animal welfare, due to frequent treatment failures, prolonged illness duration and increased mortality (Pell et al., 2025).

International institutions including the WHO and the Centers for Disease Control and Prevention (CDC) have formally recognized the antibiotic resistance as an urgent public health crisis (Aslam et al., 2018; WHO, 2022a). According to recent estimations, resistant infections were responsible for a total of 4.95 million deaths in 2019, while the O'Neill review projected an increase of up to 10 million deaths annually by 2050 in the absence of effective mitigation actions (Murray et al., 2022; O′Neill, 2016).

b. Economic losses

Beyond health threats, antibiotic resistance further imposes significant economic pressures on the healthcare system, agricultural sector and the global economic productivity. Resistant infections require higher treatment expenses and longer hospitalization periods, estimated at $29000 and 8 million additional hospital days by patient annually (Aslam et al., 2018). Furthermore, it was projected that the widespread dissemination of antimicrobial resistance could lead to a worldwide cumulative economic burden of $100 trillion and a global domestic product loss of up to 3.8% by 2050, far exceeding the cost of immediate interventions (O'Neill, 2015; WHO, 2022b).

II. Resistance to critically important β-lactams

II.1. Overview of β-Lactam antibiotics

II.1.1. Definition

β-lactam antibiotics are the most extensively used antimicrobial agents worldwide, accounting for approximately 60 % of the global antibiotic prescriptions in both human and veterinary sectors (Kim et al., 2023). These drugs are structurally characterized by the presence of a four-membered β-lactam ring, responsible for the bactericidal activity of the molecule (Mora-Ochomogo & Lohans, 2021). The β-lactam ring is usually joined with an additional heterocyclic ring in penicillins, carbapenems, and cephalosporins, forming a bicyclic structure, while monobactams only have a monocyclic core structure (Figure 3) (De Angelis et al., 2020; Kim et al., 2023).

The wide prescription of β-lactam antibiotics is mainly attributed to their efficiency, broad activity, and low toxicity (Bush & Bradford, 2016). However, this widespread use has led to the emergence and the dissemination of resistant strains, threatening the efficacy of these agents. Consequently, the WHO has categorized third- and later-generation cephalosporins and carbapenems as "Highest Priority Critically Important Antimicrobials," with exclusive regard of carbapenems for human use only, underlining the high value of these β-lactam classes for public health (WHO, 2024b).

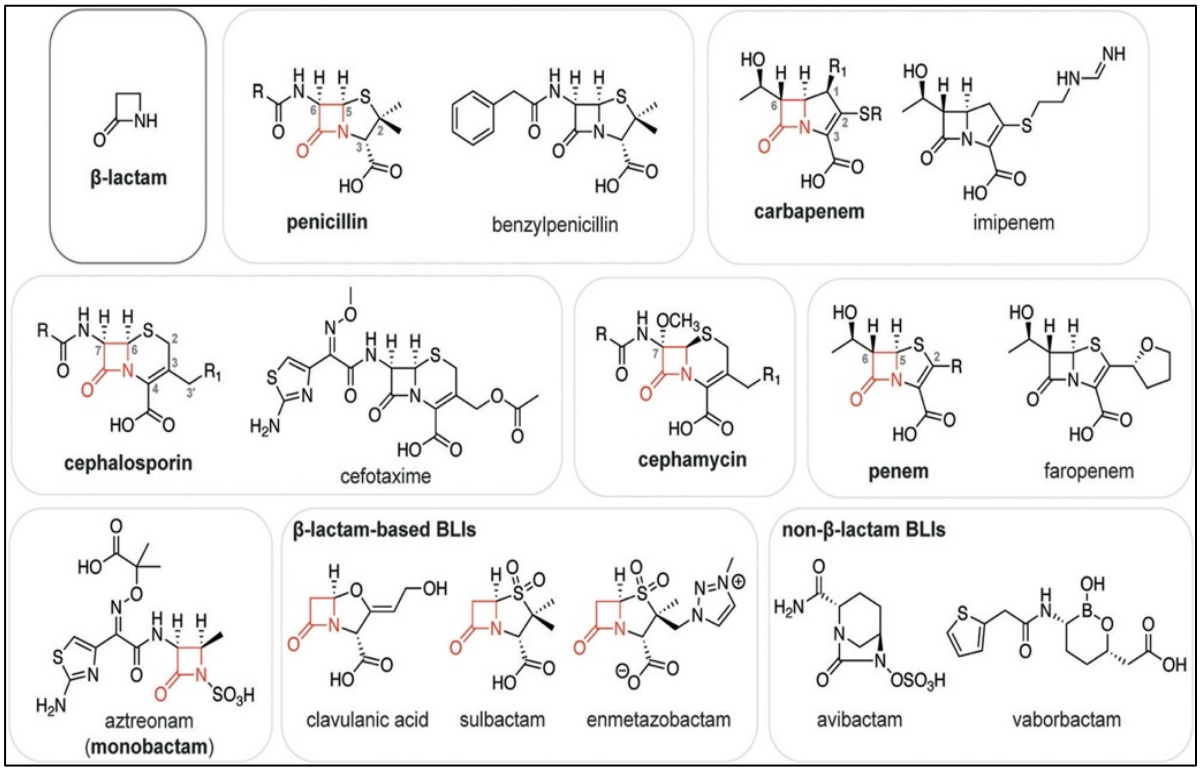

Figure 3: Structure of the β-lactam ring and the main β-lactam classes (Mora-Ochomogo & Lohans, 2021).

II.1.2. Mode of action

The bactericidal activity of β-lactam drugs results from the disruption of the bacterial cell wall synthesis. They bind specifically to penicillin-binding proteins (PBPs), which are key bacterial enzymes, mainly transpeptidases, involved in the terminal phase of the peptidoglycan synthesis (Bush & Bradford, 2016; Mora-Ochomogo & Lohans, 2021).

Following their binding to the PBPs' active site, β-lactams form a stable covalent complex that irreversibly inhibits the enzymatic activity of the PBPs and prevents the transpeptidation, causing the interruption of the peptidoglycan synthesis, which thus weakens the cell wall integrity and creates an osmotic imbalance leading to bacterial death (Brooks & Jawetz, 2013; Halawa et al., 2024).

Importantly, the action of β-lactams on the peptidoglycan results in their broad-spectrum activity against both Gram-negative and Gram-positive bacteria, as well as their selective toxicity to the bacterial cell without harming the host, since mammalian cells lack peptidoglycan (Brooks & Jawetz, 2013; Bush & Bradford, 2016).

II.2. Resistance against β-lactam antibiotics

II.2.1. Emergence of β-lactam resistance

The extensive use, and often misuse, of β-lactam in human and veterinary medicine has been immediately accompanied by the emergence of resistant strains among both Gram-negative and Gram-positive species, compromising the effectiveness of these crucial drugs. Of particular relevance, the rapid emergence and dissemination of third-generation cephalosporin- and carbapenem-resistant Enterobacterales has been declared by the WHO as a critical threat to public health. (WHO, 2024a)

II.2.2. Mechanisms of β-lactam resistance

Resistance against β-lactams in *Enterobacterales* is mediated by several, often combined, mechanisms: (i) target site alteration through structural modification of PBPs, (ii) reduction of outer membrane permeability via porin loss or modification, (iii) drug elimination via efflux pump activation, and most commonly (iv) enzymatic inactivation of the antibiotic, which involves specific enzymes hydrolyzing the β-lactam ring called β-lactamases (De Angelis et al., 2020; Kim et al., 2023).

a. Definition of β-lactamase enzymes

β-lactamases are ancient bacterial enzymes produced by multiple Gram-negative and Gram-positive species that act by hydrolyzing the β-lactam ring, which prevents the drug from binding to the PBPs and thus abolishes its antimicrobial activity (Figure 4) (Brooks & Jawetz, 2013; Bush, 2018; Halawa et al., 2024). These enzymes can be encoded either on chromosomal genes or on mobile genetic elements, including plasmids and transposons (Brooks & Jawetz, 2013; Kim et al., 2023).

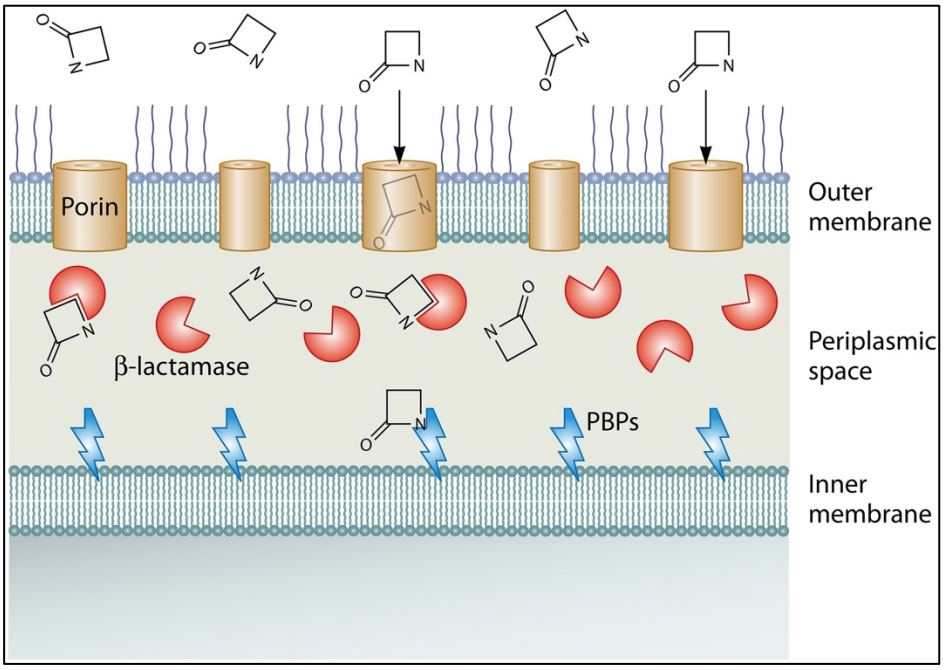

Figure 4: Mechanism of action of β-lactamase enzymes (Bush & Bradford, 2020).

b. Classification of β-lactamase enzymes

β-lactamase enzymes are classified according to two complementary criteria: structural and functional (Bush & Bradford, 2020). Structural classification was established by Ambler in 1980 based on amino acid sequences and divided β-lactamases into four distinct classes: A, B, C and D (Bush, 2018). These classes differ biochemically by their hydrolytic mechanism and are further classified into two families: serine β-lactamases and metallo-β-lactamases (Figure 5) (Bush, 2018). Class A, C and D are serine β-lactamases, which act by forming an acyl-enzyme covalent complex between the β-lactam ring and their active-site serine, while class B enzymes are metallo-β-lactamases, which require one or two zinc ions in their active site to hydrolyze the β-lactam antibiotic (De Angelis et al., 2020).

The functional classification was proposed by Bush et al. in 1995 and detailed by Bush and Jacoby in 2010. It further classifies β-lactamase enzymes into three major groups based on their biochemical properties, including substrate profiles, hydrolysis rates and susceptibility to inhibitors (Bush & Bradford, 2020). This classification encompasses group 1, which are AmpC cephalosporinase enzymes belonging to the Ambler class C, group 2 that consists of the classes A and D, including broad-spectrum β-lactamases, inhibitor-resistant β-lactamases, extended-spectrum β-lactamases (ESBL) and serine carbapenemases; and lastly group 3 that includes class B metallo-β-lactamases. Several subgroups of the three major groups were also described

based on specific characteristics of each enzyme (e.g., 1e, 2a, 2be, 3a, 3b, …) (Bush & Jacoby, 2010).

Certain β-lactamase families belong to more than one structural or functional group. For instance, ESBL enzymes can include enzymes from classes A, C, or D, corresponding to functional groups 1e, 2be, 2de, and 2e. Similarly, carbapenemases enzymes may belong to structural class A, B, or D, or to functional groups 2df, 2f, or 3 (Bush & Bradford, 2020).

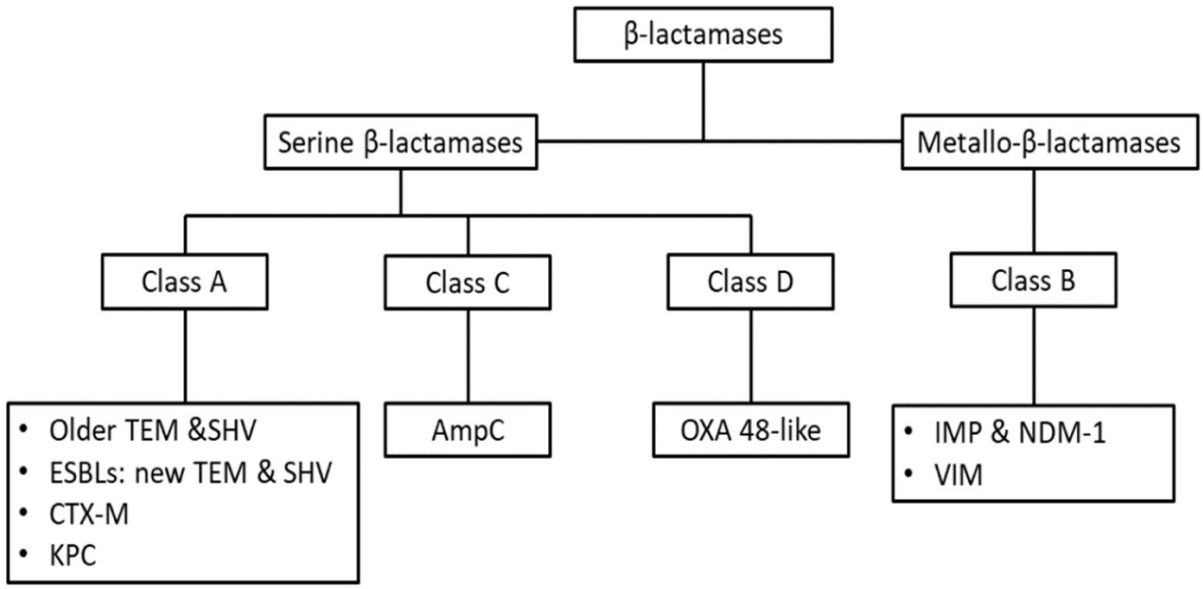

Figure 5: Classification of β-lactamase enzymes (Kakoullis et al., 2021).

c. Critical β-lactamases

- **Extended-spectrum β-lactamases (ESBLs)**

ESBLs are plasmid-mediated serine enzymes that belong to the Ambler class A β-lactamases, which were first reported in 1983 and originated through point mutation from old broad-spectrum β-lactamases (SHV-1, TEM-1, and TEM-2) (Meini et al., 2019; Rebbah et al., 2018). These enzymes are capable of hydrolyzing penicillins, cephalosporins, and monobactams but are inactive against carbapenems and cephamycins and are generally inhibited by β-lactamase inhibitors (e.g., clavulanic acid, tazobactam, and sulbactam) (Husna et al., 2023; Rebbah et al., 2018; WHO, 2022a).

ESBL enzymes are the main β-lactam-resistance mechanism in Enterobacterales. (Rebbah et al., 2018) They are most frequently produced by *E. coli* and *Klebsiella species* but can be found in other Gram-negative bacteria (e.g., *Pseudomonas* and *Morganella*) (Girlich et

al., 2020). Currently, the CTX-M enzymes are the most prevalent ESBL worldwide; this enzyme has drastically shifted the epidemiology of ESBLs due to its widespread and rapid dissemination across different bacterial hosts and ecological niches (Hayer et al., 2022).

ESBL-encoding genes exhibit a high genetic diversity, they are mainly carried on plasmids and transposons, often with other resistance genes, such as aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole, and thus contribute to the spread of multidrug-resistant phenotypes (Husna et al., 2023; Ibekwe et al., 2021).

- **AmpC β-lactamases**

AmpC are ancient chromosomal inducible enzymes produced by multiple bacterial species, they were first described in 1988 and belong to the Ambler class C serine β-lactamases (Bush & Bradford, 2020; Girlich et al., 2020; Meini et al., 2019). These enzymes are active against penicillins, cephalosporins, cephamycins, oxyimino-cephalosporins, and monobactams and are not susceptible to clavulanic acid, tazobactam, or sulbactam but are inhibited by avibactam, vaborbactam, and relebactam (Bush & Bradford, 2020).

AmpC-encoding genes are harbored on chromosomes of multiple *Enterobacterales* species such as *Enterobacter cloacae*, *Serratia marcescens,* and *Citrobacter freundii*, but they can also be mobilized onto plasmids and therefore transferred to other species such as *E. coli* and *K. pneumoniae,* which normally lack chromosomal AmpC genes, making their mitigation even more challenging (Meini et al., 2019).

- **Carbapenemases**

Carbapenemases are β-lactamase enzymes that belong to Ambler classes A, B, and D and that are potent against a broader spectrum of β-lactam antibiotics, including penicillins, cephalosporins, monobactams, and carbapenems. They are produced by multiple Enterobacterales species and other Gram-negative bacteria (Bonomo et al., 2018; WHO, 2022a).

Class A serine carbapenemases are mainly represented by Klebsiella pneumoniae carbapenemase (KPC) in addition to other enzymes (e.g., IMI, GES, and NMC) and are generally inhibited by clavulanic acid and boronic acid. Class B metallo-β-lactamases, including NDM, IMP, and VIM enzymes, are active against all β-lactam drugs except

aztreonam and are inhibited by EDTA. Finally, carbapenemases of class D, including OXA-type, are serine enzymes that hydrolyze carbapenems but exhibit weak or no activity against cephalosporins, they are not inhibited by standard β-lactamase inhibitors (Lee et al., 2022; Touati & Mairi, 2019).

Carbapenemase enzymes are mainly encoded on conjugative plasmids, facilitating their broad dissemination across different bacterial species and reservoirs, including humans, animals, and the environment (Touati & Mairi, 2019).

II.2.2. Dissemination of β-lactamase-producing bacteria among livestock

β-lactamase enzymes are widely disseminated across various ecological niches, including food-producing animals, raising serious public health concerns. This widespread is mostly mediated by mobile genetic elements such as plasmids facilitating the circulation and the persistence of β-lactamase-encoding genes in the farm environment and their transmission to humans via multiple routes (Madec et al., 2017).

Among β-lactamase enzymes, ESBLs are the most frequently detected in livestock, with CTX-M-type showing the highest rate (Madec et al., 2017). These enzymes are predominantly reported in poultry and pigs, which are considered the main livestock reservoirs of ESBLs (Silva et al., 2023). They were also reported in cattle and small ruminants in several countries, but at lower rates (Ben Haj Yahia et al., 2023; Tello et al., 2020).

AmpC enzymes, mainly CMY and DHA, are also broadly disseminated among food-producing animals, including poultry, pigs, cattle, and sheep, but still less frequent than ESBLs (Collis et al., 2022; EFSA & ECDC, 2025; Tello et al., 2020). Interestingly, the co-occurrence of plasmid-mediated AmpC and ESBL in Enterobacteriaceae from animal sources has been widely reported (Belmahdi et al., 2016; Homeier-Bachmann et al., 2022). This co-selection, mainly attributed to the extensive use of 3GC, represents a major threat to the public health as it compromises the efficacy of most β-lactams and restricts available therapeutic options (ECDC et al., 2021).

In contrast to ESBL and AmpC, carbapenemase enzymes are less common in animals, since the use of carbapenem antibiotics in the veterinary sector is unallowed (Ramírez-Castillo et al., 2023). However, carbapenemase-producing bacteria are increasingly emerging and

disseminating in food-producing animals. They were detected in poultry, cattle, small ruminants, and pigs worldwide (Carfora et al., 2022; Hamza et al., 2016; Tello et al., 2020). The propagation of carbapenemase-producing strains and/or their plasmids in farm animals may result from direct human-animal contact, environmental contamination, or further derive from the selective pressure exerted by 3GC use in livestock (Bonardi & Pitino, 2019). While still rare, the detection of carbapenemases in livestock threatens a crucial last-resort antibiotic class and raises significant concerns for both animal and human health.

III. Colistin resistance

III.1. Colistin overview

III.1.1. Definition

Colistin, also known as polymyxin E, is a polycationic peptide produced as a fermentation product by *Paenibacillus polymyxa* subsp. *Colistinus* (Caniaux et al., 2017; El-Sayed Ahmed et al., 2020). The polymyxin antibiotics are composed of a cationic polypeptide attached to a lipophilic fatty acyl side chain (Hussein et al., 2021). This family includes five compounds, polymyxin A, B, C, D and E. However, only polymyxin B and E (colistin) are clinically applied (Figure 6) (Hussein et al., 2021).

Colistin was first used in Japan and Europe in the 1950s (Poirel et al., 2017). Later, it was approved by the Food and Drug Administration agency (FDA) in 1959 and was excessively applied in human and veterinary medicine for the treatment of infections caused by Gram-negative bacteria, as well as the enhancement of animal growth (El-Sayed Ahmed et al., 2020). Yet, the colistin use was prohibited in the 1970s because of its nephrotoxicity and neurotoxicity and was only restricted for cystic fibrosis treatment (Poirel et al., 2017). In the 1990s, colistin was reintroduced as a last-line antibiotic as a result of the increasing emergence of multidrug-resistant Gram-negative bacteria (El-Sayed Ahmed et al., 2020).

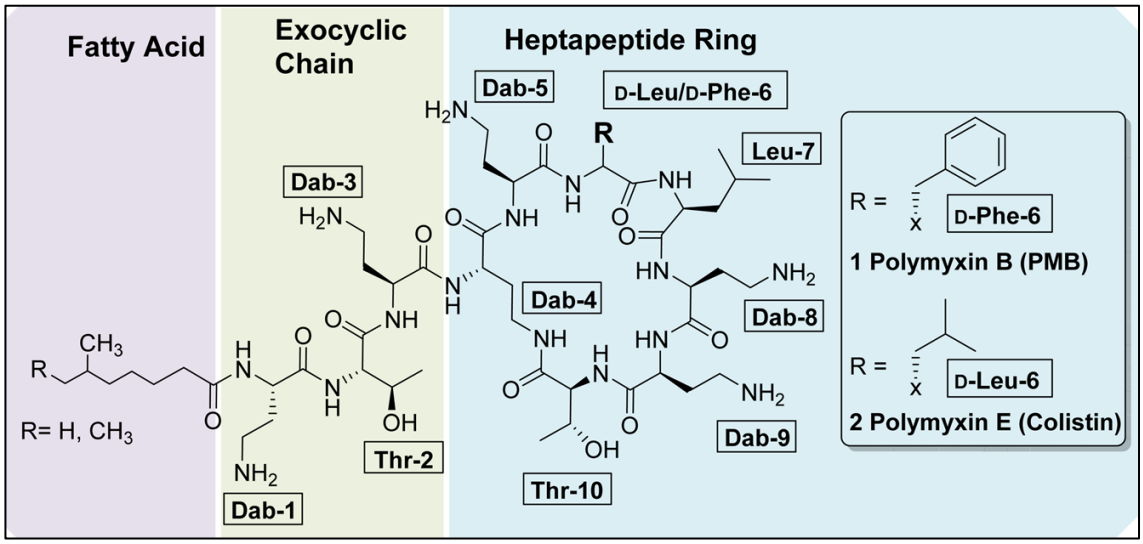

Figure 6: Structure of polymyxins (Gallardo-Godoy et al., 2016).

III.1.2. Mechanism of action

The colistin activity is based on an electrostatic interaction between the cationic antibiotic and the lipid A of the lipopolysaccharide (LPS), which has a negative charge (Apostolakos & Piccirillo, 2018; Moffatt et al., 2019). After its binding to the LPS on the outer membrane of Gram-negative bacteria, colistin disrupts the membrane organization by displacing the divalent cations on the LPS, leading to the leakage of the cell content and thus the bacterial death (Figure 7) (Apostolakos & Piccirillo, 2018; Hussein et al., 2021).

However, this mechanism narrows the colistin spectrum only to the Gram-negative bacteria, due to the absence of the LPS in the Gram-positive bacterial membrane (Apostolakos & Piccirillo, 2018). Additionally, some Gram-negative bacteria as *Serratia spp* and *Proteus spp* are naturally resistant against this agent (Apostolakos & Piccirillo, 2018).

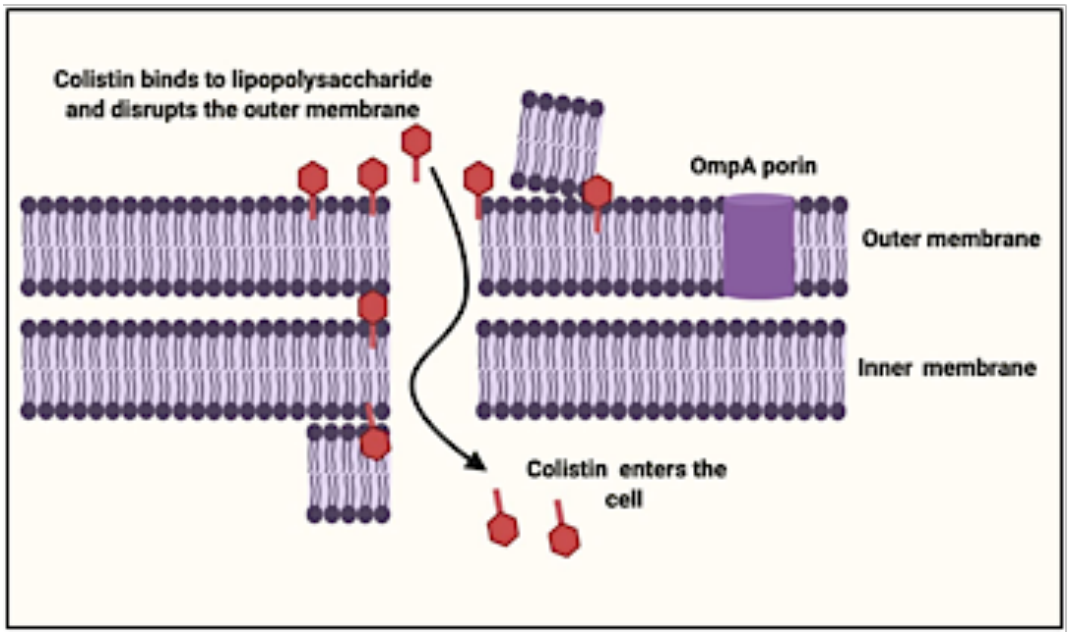

Figure 7: Mechanism of action of colistin (Hussein et al., 2021).

III.2. Mechanisms of resistance against colistin

III.2.1. Chromosomally mediated colistin resistance

The extensive use of colistin has led to the emergence of resistant strains and their spread across different niches. Colistin resistance was initially attributed to intrinsic chromosomal modifications in the bacterial genome (Chatzidimitriou et al., 2022). The most frequent resistance mechanisms involve the modification of the LPS via the addition of phosphoethanolamine (PEtn), galactosamine and/or 4-amino-L-arabinose (L-Ara4N) (Moffatt et al., 2019). Additionally, other intrinsic modifications have been shown to provide resistance against colistin, including the complete loss of the LPS, capsular polysaccharide overproduction, efflux pump overexpression, and porin mutations (El-Sayed Ahmed et al., 2020).

III.2.2. Plasmid-mediated colistin resistance

a. Emergence

In late 2015, Liu and his colleagues revealed the presence of a new colistin-resistance mechanism encoded by a plasmid-borne gene, the *mcr-1*, that was carried by an *Escherichia*

coli isolated from a pig in China (Liu et al., 2016). Yet, a retrospective study has reported an earlier emergence of this gene in Chinese poultry dating back to the 1980s (Shen et al., 2016).

The discovery of *mcr-1* has shed light on the alarming concern of the rapid dissemination of colistin resistance via plasmids, imposing a critical threat to public health. Over the years, nine homologs of the *mcr-1* were identified (*mcr-2* to *mcr-10*) and over 22 variants were reported (Chatzidimitriou et al., 2022; Liu et al., 2024). It has been suggested that *mcr-1* and *mcr-2* genes originated from intrinsic *mcr*-like genes carried on the chromosomes of *Moraxella* species, while the other *mcr* genes have probably emerged from the chromosomes of *Aeromonas* species, *Buttiauxella*, *Kosakonia*, *Shewanella*, and more (Table 2) (Liu et al., 2024).

The *mcr* genes have been identified more than 27 bacterial hosts, with 91% of *mcr*-carrying isolates identified as *E. coli*, followed by *Salmonella* enterica (7%) and *Klebsiella pneumoniae* (2%) (Elbediwi et al., 2019; Mondal et al., 2024). Other Gram-negative bacteria, namely *Enterobacter* species, *Cronobacter sakazakii*, *Moraxella*, *Proteus mirabilis*, *Shigella sonnei* and *Aeromonas*, have also been reported as *mcr*-carriers but at a lower rate (Mondal et al., 2024). Additionally, it has been reported that the *mcr-1* gene can be transmitted to *Pseudomonas aeruginosa* by conjugation (Hussein et al., 2021).

The *mcr-1* gene was first detected on an IncI2 plasmid, and later, various plasmid types bearing *mcr* genes have been reported, with IncI2, IncHI2 and IncX4 being the most predominant ones (El-Sayed Ahmed et al., 2020) (Liu et al., 2024). IncI2 and IncX4 are deemed "epidemic plasmids" owing to their ability to carry and spread *mcr-1* gene globally among *Enterobacterales* from human and animal sources (Mondal et al., 2024).

Table 2: *mcr* genes, origin and plasmids (Liu et al., 2024).

Genes	Origin	Main plasmids
mcr-1	*Moraxella* species	IncX4, IncI2, IncHI2, IncA, IncF, IncHI1, IncHI2, IncI1, IncK, IncN, IncQ, IncX1
mcr-2	*Moraxella* species	IncHI1B/IncFIB, IncX4

mcr-3	*Aeromonas* species	IncHI2, IncF, IncFIA, IncFII, IncHI2/IncY, IncN, IncP, IncR, IncX1, IncY, IncF
mcr-4	*Shewanella* species	ColE, ColE10
mcr-5	-	ColE10, IncFI, IncFII, IncHI2, IncI1, IncN, IncP, IncX1
mcr-6	-	ColE
mcr-7	*Aeromonas* species	ColE-like, IncI2
mcr-8	*Kosakonia* species	IncA/C, IncFIA, IncFII, IncFIIK, IncQ, IncR
mcr-9	*Buttiauxella* species	IncHI2, IncFI, IncFII, IncI1-I, IncI2, IncN, IncP
mcr-10	*Buttiauxella* species	IncF, IncFIB, IncFII

b. Structure and mechanism of action of MCR enzymes

Structurally, MCR enzymes are phosphoethanolamine (pEtN) transferases, featuring both catalytic (C-terminal) and transmembrane (N-terminal) domains and belonging to the YhjW/YkdB/YijP alkaline phosphatase superfamily (Liu et al., 2024). Phylogenetic comparison showed that the MCR-1 enzyme shares high homology with MCR-2 and MCR-6 in terms of amino acid sequence, forming one subgroup, while the other variants belong to separate subgroups owing to their distinct amino acid composition (Liu et al., 2024).

The MCR enzymes catalyze the transfer of the phosphoethanolamine to the lipid A reducing its negative charge and thus decreasing the colistin binding affinity to the membrane of the bacterial host (Mondal et al., 2024). This mechanism is ensured by the transmembrane and the catalytic domains of the enzyme: the N-terminal domain of the MCR fixes the enzyme in the inner membrane of the bacteria, while the C-terminal domain transfers the pEtN to the lipid A of the LPS leading to the reduction of the colistin affinity to the membrane and compromising its efficiency (Figure 8) (El-Sayed Ahmed et al., 2020).

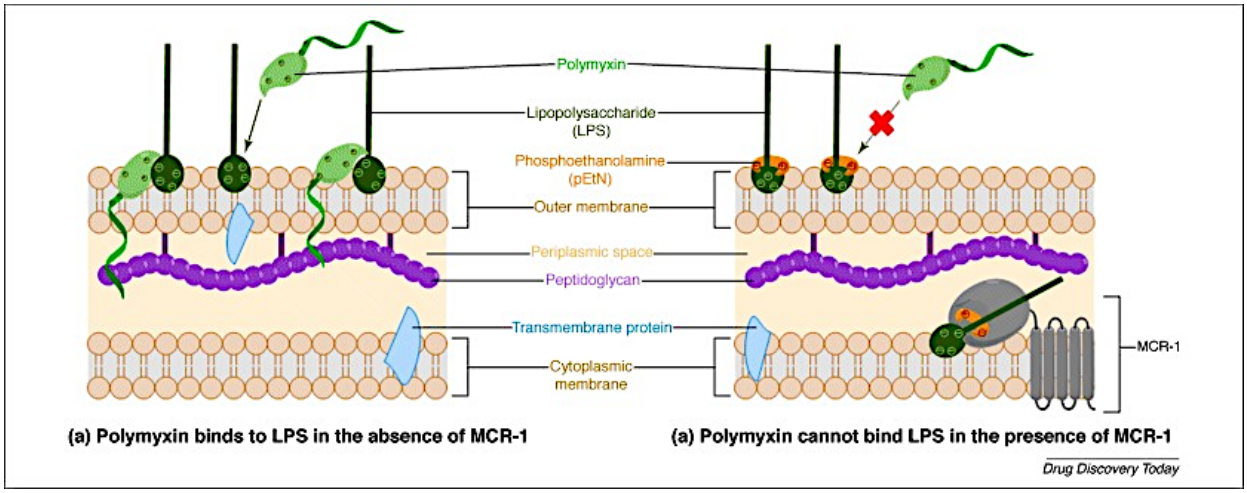

Figure 8: Mechanism of MCR-mediated colistin resistance. (Son et al., 2019)

III.3. Dissemination of *mcr*-carrying bacteria among livestock

mcr-carrying bacteria have disseminated among diverse ecological niches, with a higher prevalence in livestock, underscoring the key role of food-producing animals as a reservoir for this resistance (Rhouma et al., 2016). Indeed, epidemiological data indicated that mobile colistin resistance is believed to be originated and transmitted to humans from animals through direct and indirect routes. (Apostolakos & Piccirillo, 2018; Mmatli et al., 2022)

The extensive use of colistin in animal husbandry likely contributes to the selection of resistant strains and their persistence in the farm environment (Khine et al., 2022). Furthermore, the widespread dissemination and stability of *mcr* genes is associated with their carriage on transferable plasmids of different sizes and incompatibility groups and which are highly conjugative facilitating the spread of *mcr* genes among different niches (Mmatli et al., 2022; Mondal et al., 2024).

IV. Addressing antibiotic resistance in livestock through a One Health framework

IV.1. Definition and origin of the “One Health” concept

The « One Health » notion represents a collaborative cross-sectoral framework that underscores the interconnectedness between human, animal, and environmental health (WHO, 2017b). This approach recognizes that complex health challenges like antibiotic resistance must be addressed in conjunction within an integrated strategy that combines multiple

disciplines, such as human and veterinary medicine, environmental science, and other related fields (McEwen & Collignon, 2018; WHO, 2017b).

Even though the interdependence between human, animal and environmental reservoirs has long been acknowledged, the concept of One Health was officially introduced in 2004 at the conference of Wildlife Conservation Society under the initiative "One World, One Health" (Panda et al., 2021). Later, this approach was progressively adopted by international associations, including the WHO, the FAO and the WOAH. As a result of this global recognition, several national and international surveillance programs have been established in order to address health concerns through an integrated framework that unites human, animal and environmental health sectors.

IV.2. Role of the One Health concept in combating antibiotic resistance in livestock

The increasing emergence and rapid dissemination of antibiotic-resistant bacteria among food-producing animals emphasizes the urgent need for the One Health framework. Indeed, the antibiotic agents used in livestock production are usually identical or close to those used in human medicine. Therefore, the extensive use and misuse of these agents in the veterinary sector threatens the efficiency of antimicrobial therapy in both human and veterinary medicine, as it promotes the selection of resistant bacteria in the farm environment and eventually their spread to other ecological niches, including humans (WHO, 2017b).

The One Health approach plays a multi-dimensional role in addressing the global challenge of antibiotic resistance by providing a unified framework for surveillance, stewardship and interventions (FAO, 2021; WHO, 2017a). From a practical perspective, the One Health strategy allows the identification of critical control points that can be targeted in order to minimize antibiotic resistance risks, such as monitoring antibiotic use in agriculture, controlling environmental contaminations and advocating alternatives for antibiotics in animal production (WHO, 2017a).

IV.3. Integrated One Health action plan against antibiotic resistance

Within the tripartite collaboration, the WHO, the FAO and the WOAH have implemented an integrated action plan consisting of five strategic objectives that aim to mitigate antibiotic resistance through improving awareness, surveillance, prevention, rational

use and sustainable investment. These objectives collectively guide countries to develop and implement their own national action plan in line with the global strategy in order to promote cross-sectoral collaboration against antibiotic resistance within the One Health framework (WHO, 2015).

IV.3.1. Improving awareness and understanding of antibiotic resistance

Raising awareness about antibiotic resistance is a fundamental and immediate action in the global mitigation strategy. This objective can be achieved through targeted public communication programs designed to increase the understanding of antibiotic resistance and its risks among different audiences involved in human health, veterinary medicine, agriculture and food production as well as consumers and general public (WHO, 2015; WOAH, 2016).

Furthermore, incorporating the use of antibiotic agents and the notion of resistance in school programs will promote the awareness from an early age, while integrating the understanding of antibiotic resistance in professional education and training certificates will ensure proper knowledge among professionals including medical staff, veterinarians and farmers (WHO, 2015).

IV.3.2. Strengthening surveillance of antibiotic resistance

Integrated surveillance is a vital component of the One Health strategy against antibiotic resistance. This approach has been explicitly advocated by global organizations in official policy documents, such as the WHO Global Action Plan for AMR, the WOAH strategy for AMR and the prudent use of antimicrobials, and the FAO action plan on AMR (FAO, 2021; WHO, 2015; WOAH, 2016).

Surveillance systems aim to monitor antibiotic consumption and resistance patterns in humans, animals and the environment, detecting newly emerged resistance and understanding the roots of development and circulation of resistant strains within and between ecological niches, in order to provide essential data for policy making and stewardship interventions (Léger et al., 2022; WHO, 2015).

IV.3.3. Prevention and control of antibiotic-resistant infections through good practices

The prevention and control of infections is a key strategy to reduce the need for antibiotic treatment and thus mitigate the resistance. This objective aim to limit the emergence and the propagation of resistant bacteria by improving biosecurity measures and sustainable practices in healthcare settings and animal husbandry (FAO, 2021; WHO, 2015).

The vaccination, where appropriate, is an effective approach to reduce bacterial infectious diseases requiring antibiotic treatment or viral infections often improperly treated with antibiotics and eventually promoting the incidence of secondary bacterial infections (WHO, 2015). Regarding the agriculture sector, appropriate farming and production practices including rational use of antibiotics, proper sanitation and hygiene measures, adequate effluent treatment and strategic vaccination can lower infections rate and improve animal welfare which will minimize the need for antibiotic consumption and reduce resistance development and spread (FAO, 2021).

IV.3.4. Optimizing the use of antibiotics in human and veterinary medicine

Advocating the prudent use of antibiotics in the human and veterinary sectors is a crucial approach in limiting antibiotic resistance. The primary step for this objective is diffusing the public recognition of antibiotic agents in order to enhance the regulation of their production, distribution, quality and proper use (WHO, 2015). Training professionals on the standard guidelines of antibiotic prescription will improve the proper application of these agents and tackle their misuse (FAO, 2021).

Facilitating access to effective, rapid and affordable diagnostic and prescription is needed to ensure the rational use of antibiotic in both human and veterinary medicine (FAO, 2021). Likewise, the prescription and dispense of antibiotic drugs should be performed by health professionals and exclusively through an evidence-based diagnosis (WHO, 2015). The withdrawal of unnecessary use of antibiotics in agriculture such us animal growth promoters and plant pesticides will help reducing the spread antibiotic resistance in animal farming and food chain (FAO, 2021).

IV.3.5. Increasing governance, investment and sustainability

The effective mitigation of antibiotic resistance requires a multisectoral engagement aiming to ensure coordinated national efforts and sustainable investment. This objective consists of strengthening national policies and regulatory frameworks to ensure a rapid and effective action against antibiotic resistance (FAO, 2021; WOAH, 2016).

To attain this objective, global organizations aim to support member countries to develop and implement policies for the governance of antibiotic use in human and veterinary sectors while providing guidance and assistance in their initiatives against antibiotic resistance (WOAH, 2016). Furthermore, investing in research and development of therapeutic alternatives, vaccines and diagnostic tools as well as rapid and affordable antibiotic susceptibility testing in all healthcare and veterinary settings is required to mitigate the excessive use of antibiotic agents (FAO, 2021; WHO, 2015). Nonetheless, the applicability of this strategy requires the assessment of the global socioeconomic impact of antibiotic resistance and evaluating the cost-effectiveness of action against the inaction (WHO, 2015).

IV.4. Global and national One Health initiative against antibiotic resistance

Several national, regional and international programs and initiatives have been implemented in accordance with the global action plan promoted by the WHO, WOAH and FAO, aiming to harmonize reporting methodologies across countries and ensure the data comparability within a One Health framework (Table 3).

Table 3: Examples of One Health initiatives on antibiotic resistance

Program	Region/Country	Year of foundation	Aim	Reference
The WHO Global Action Plan on antimicrobial resistance	Global	2015	Preserve the effectiveness of existing antimicrobials and ensure their accessibility and appropriate use	(WHO, 2015)

The OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials	Global	2016	Preserve the efficacy of antimicrobial agents and ensure their prudent use in animals	(WOAH, 2016)
The FAO Action Plan on antimicrobial resistance	Global	2021	Promoting responsible use of antimicrobials and tackling resistance in the agriculture sector	(FAO, 2021)
The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC)	European Union	2009	Collection and report of data on antimicrobial usage data in the veterinary sectors	(EMA, 2023)
Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP)	Denmark	1995	Collection of data on antimicrobial use and resistance in humans, animals and food	(DANMAP, 2022)
Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS)	Canada	2002	Collection and analysis of data on antimicrobial use and resistance in human, animals and food.	(Government of Canada, 2015)

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands (MARAN)	Netherlands	1998	Monitor antimicrobial resistance and antimicrobial usage in animals	(Veldman et al., 2019)

Methods

1. Study design and farm selection

This study aimed to investigate the dissemination of colistin-resistant and 3GC-resistant *E. coli* in poultry, bovine and ovine farms. It was carried out between September 2021 and December 2022 in Guelma province, northeast of Algeria, among six regions: Heliopolis (7 bovine and 2 poultry farms), Bouati Mahmoud (11 bovine, 6 ovine and 1 poultry farm), Hammam Debagh (3 bovine farms), Bendjerrah (2 poultry farms), Medjez Ammar (3 poultry and 1 ovine farm) and Guelaât Bou Sbaâ (2 poultry farms). The selection of farms was based on their accessibility (Figure 9).

Bovine farms sampled in Heliopolis and the ovine farm sampled in Medjez Ammar are located in urban regions, while all the other farms are distributed in rural zones.

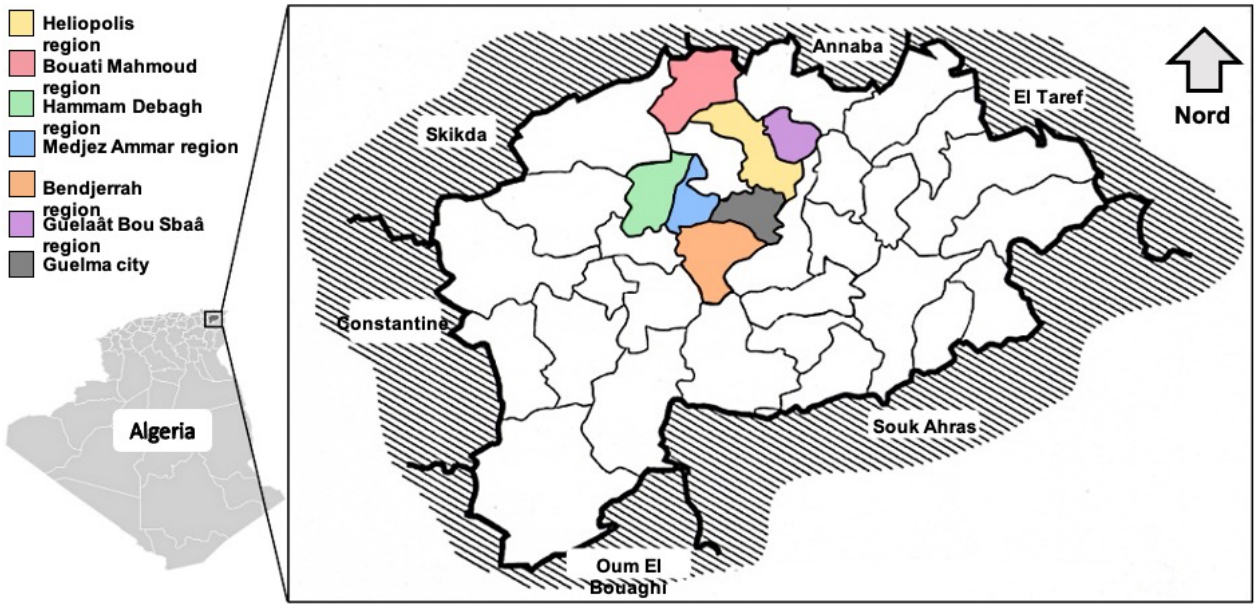

Figure 9: Geographic location of the sampled regions.
(https://dcwguelma.dz/fr/index.php/recueils-des-textes/10-menu-principal)

1.1. Poultry farms:

A total of 10 poultry farms, F01.p to F10.p, were sampled. They were all managed under intensive farming system and were distributed among 5 regions: Heliopolis (n=02), Medjez Ammar (n=03), Bendjerrah (n=02), Guelaât Bou Sbaâ (n=02) and Bouati Mahmoud (n=01) (Table S1).

- **Longitudinal study of *mcr-1*-positive *E. coli* persistence in poultry farms:**

Based on the first sampling results, three positive *mcr-1* farms, F06.p, F07.p and F08.p, were conveniently selected to further assess the potential persistence of *mcr-1*- producing *E. coli* in these farms with a second and a third longitudinal sampling campaign.

Two of the farms were located in Bendjerrah and were about 600 m apart, while the third one was situated in Medjez Ammar at an approximative distance of 9 km from the other two farms (Figure 10).

Additional data regarding animal's health, antibiotic usage and disinfection routine were collected from farms F06.p, F07.p and F08.p for a more accurate evaluation of the persistence in the farms.

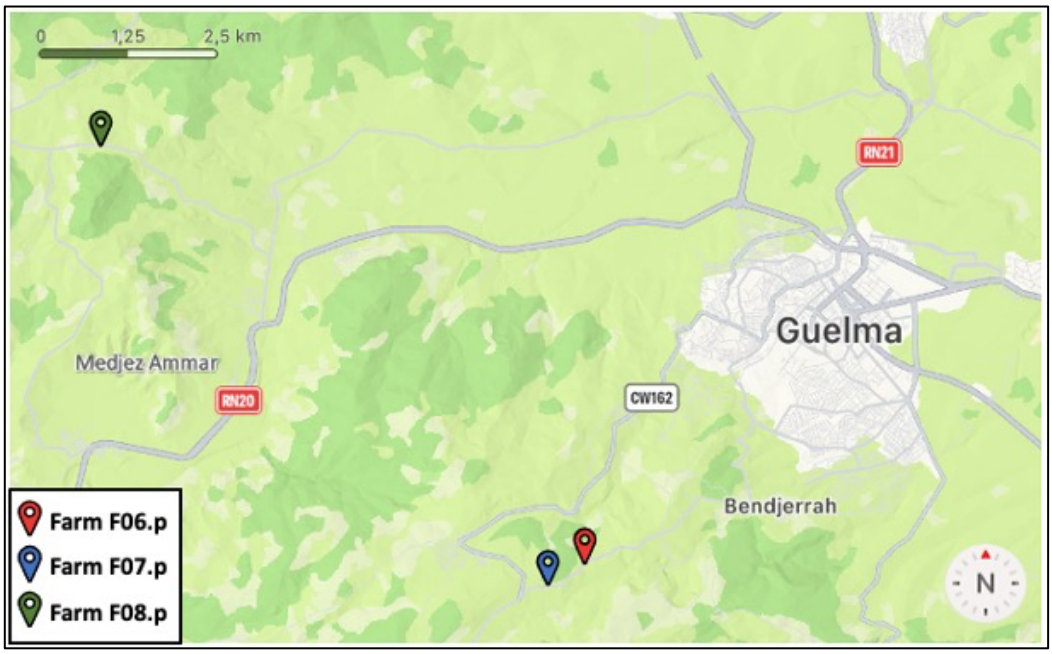

Figure 10: Geographic location of the farms included in the longitudinal surveillance. (Apple Maps, Apple Inc., 2025)

1.2. Bovine farms:

A total of 21 bovine farms coded from F11.b to F31.b were investigated. They were located in 3 regions: Heliopolis (n=07), Bouati Mahmoud (n=11) and Hammam Debagh

(n=03). Among the farms sampled in the Heliopolis region, 6 were reared under intensive systems, while all the other bovine farms were semi-extensive (Table S2).

1.3. Ovine farms:

The 7 investigated ovine farms, marked from F32.o to F38.o, were situated in Bouati Mahmoud (n=06) and Medjez Ammar (n=01) and were all managed under a semi-extensive farming system (Table S3).

2. Sample collection

2.1. Poultry sampling

Given the large number of chickens in poultry farms, samples were collected following a visionary matrix as previously described by the *Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS)*, with slight adjustments (Government of Canada, 2015). Concisely, each poultry house was visually divided into six or eight lines, according to its area. Then, 5 sampling points were equally allocated on each sampling line, giving 30 (5 points x 6 lines) or 40 (5 points x 8 lines) sampling points in every house (Figure 11).

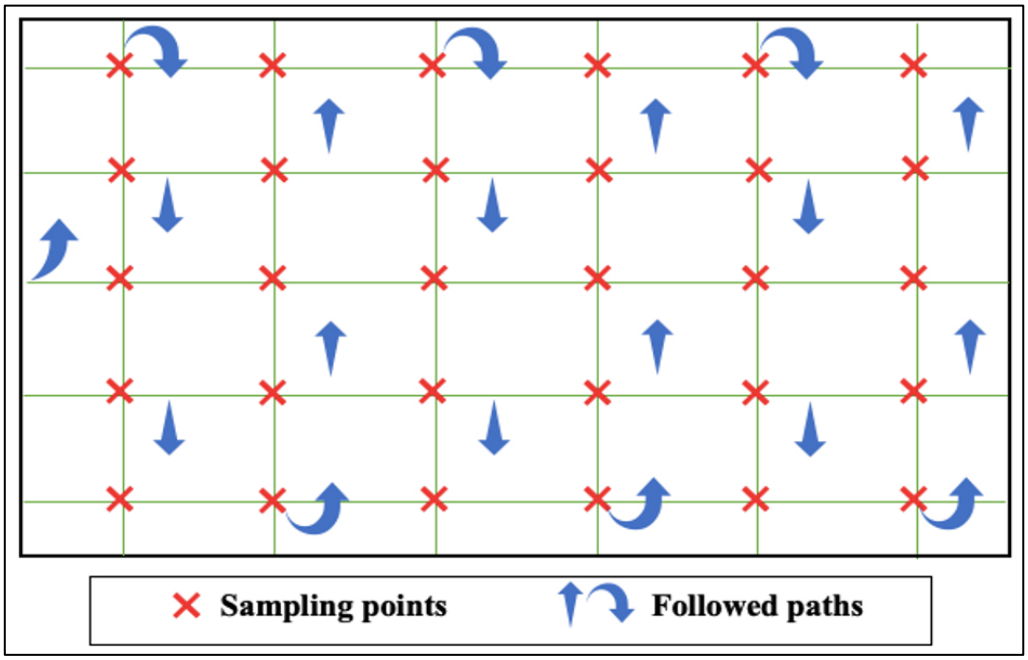

Figure 11: Sampling matrix (Example of 30 sampling points)

Fresh fecal samples were collected from each sampling point using a sterile cotton swab, without stepping on the same path twice. A total of 40 chicken droppings were collected in farm F06.p, whereas only 30 samples were gathered from the other farms due to their smaller area.

Primarily, only chicken fecal samples were collected during the first sampling campaign. However, rooster (n=02) and duck (n=02) fecal samples were exceptionally collected in farm F06.p while environmental samples were also included in farm F09.p, including animal drinking water (n=02) and soil samples (n=02).

In the subsequent sampling campaigns, animal and environmental samples were gathered from each of the three farms selected for the longitudinal surveillance (F06.p, F07.p and F08.p), including: food samples (n=02), drinking water (n=02), wastewater (n=02), soil samples (n=02) and wall swabs (n=02). In addition, rooster and duck droppings were again collected in the farm F06p (Table S1).

An exception was made for farm F08.p where only environmental samples were collected in the third campaign due to the absence of animals during this period, and therefore a fourth sampling was exclusively conducted on this farm once there was a new chicken flock.

Samples were immediately transported to the laboratory in a portable insulated cooler and analyzed within the same day to preserve bacterial viability.

2.2.Bovine and ovine sampling

Cattle and sheep fecal samples were directly collected from the accessible animals with rectal swabs. The sampling was performed by a practician veterinarian, ensuring no animals were hurt or stressed. Samples were transferred to the laboratory and processed on the sampling day (Tables S2 & S3).

3. Bacterial isolation and identification

The bacteriological analyses were carried out at the laboratory of Biology, Water and Environment (LBEE), University of 8 May 1945, Guelma

3.1. Enrichment

Bovine and ovine swabs were analyzed individually. As for chicken samples, every five swabs from the same sampling line were pooled in the same tube to constitute one sample. All samples were enriched in Mueller Hinton Broth (MHB) at 37 °C for 18 h.

3.2. Screening

Samples were separately screened for 3GC-resistant and colistin-resistant *E. coli* using selective MacConkey plates supplemented with 1 µg/mL cefotaxime or 3 µg/mL colistin, respectively (Mezhoud et al., 2015; Soria-Segarra et al., 2022).

Preparation of antibiotic stock solutions:

A 1 mg/mL stock solution for each antibiotic used for the screening was prepared as previously described by the Clinical and Laboratory Standard Institute (CLSI, 2012). Briefly, the required mass of antibiotic powder was calculated using the following formula:

$$\text{Weight (mg)} = \frac{\text{Volume (mL)} \times \text{Concentration (µg/mL)}}{\text{Potency (µg/mg)}}$$

- The final volume prepared for each stock solution is 10 ml
- The concentration is 1 mg/mL (or 1000 µg/ml)
- The potency is given by the manufacturer and corresponds to the concentration of the pure agent in the antibiotic powder.

The weighted mass was dissolved in 10 mL of sterile distilled water and vortexed, then filtered through a 0,2 µm syringe filter. Each stock solution was aliquoted into 500 µL in sterile microtubes and stored at -20 °C until use. In order to preserve the antibiotic activity, stock solutions wre not refrozen or stored for more than 6 months (NCDC, 2020).

3.3. Identification

3.3.1. Presumptive identification:

One representative lactose-positive colony was selected from each screening plate and subjected to Gram coloration and oxidase disc test. Afterwards, presumptive isolates were

plated on CHROMagar Orientation media to further screen *E. coli* isolates based on the color and appearance of their colonies (Figure 12).

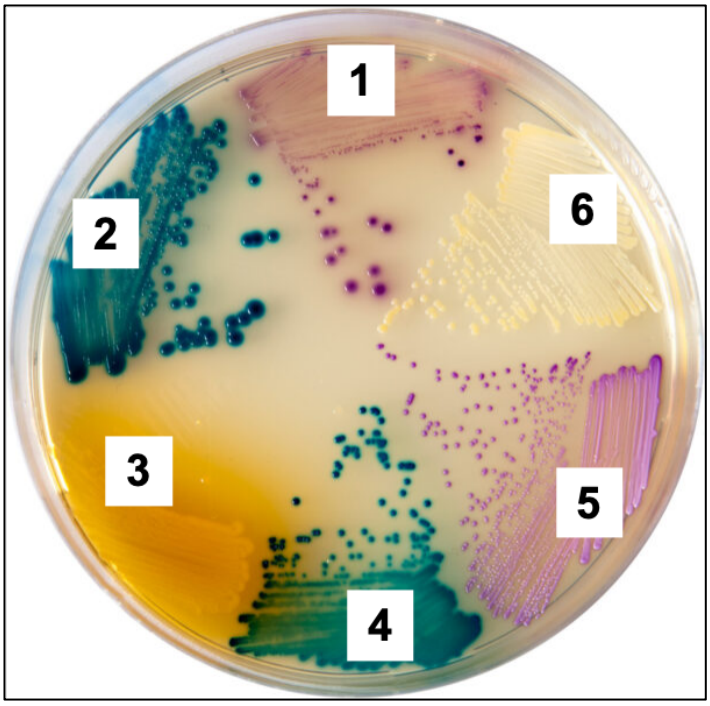

Figure 12: Bacterial colonies on CHROMagar™ Orientation (**1:** *Escherichia coli*, **2:** *Klebsiella spp., Enterobacter spp., Serratia spp.*, **3:** *Proteus spp.*, **4:** *Enterococcus spp.*, **5:** *Staphylococcus saprophyticus*, **6:** *Staphylococcus aureus*). (https://www.chromagar.com/product/chromagar-orientation/)

3.3.2. Identification with API 20 E gallery :

The API 20 E gallery (Biomérieux, France) is a miniaturized identification system that consists of 20 biochemical tests in the form of dehydrated substrate designed for the specific identification of *Enterobacteriaceae* and other Gram-negative bacteria.

Succinctly, a bacterial suspension of each isolate was prepared by transferring 3 to 5 well isolated colonies into sterile distilled water then homogenized. The suspension turbidity was adjusted to a 0.5 McFarland (≈1.5 × 10^8 CFU/mL) using a spectrophotometer set at λ = 625 nm, with an absorbance range of 0.08 – 0.10 to ensure standardization of bacterial density. Each suspension was inoculated in a gallery according to the manufacturer guidelines. After an overnight incubation at 37°C, positive and negative results were directly observed with spontaneous color changes or revealed with specific reagents.

The perceived result was coded into a 7-digit number, which was later interpreted using the identification software APIWEB™, to obtain the specific identification of the isolate.

3.3.3. Identification with MALDI-TOF mass spectrometry:

The identification of CL-R strains was further confirmed using Matrix Assisted Laser Desorption Ionization - Time of Flight mass spectrometry (MALDI-TOF MS) that was performed at the Limoge University Hospital in France. This technique identifies bacterial species based on their unique protein content profiles, which are mainly composed of highly conserved ribosomal proteins serving as optimal biomarkers owing to their stability and species-specificity (Seng et al., 2010).

Briefly, a small portion of fresh bacterial colonies were deposited on a MALDI-TOF target plate. Afterwards, 1 µL of the matrix solution was applied on each spot containing the bacterial sample and air-dried for 5 to 10 minutes at room temperature. The dried target plate was later inserted in the MALDI-TOF MS device (Vitek®MS, Biomérieux, France) and projected to laser ionization. The resulting ions were accelerated through a vacuum tube to measure their time of flight (TOF) which is in correlation with the protein mass in the samples. Finally, the mass spectrum is collected by the system and compared to the reference database in order to provide the specific identification (Figure 13) (Bizzini & Greub, 2010; Blondiaux et al., 2010).

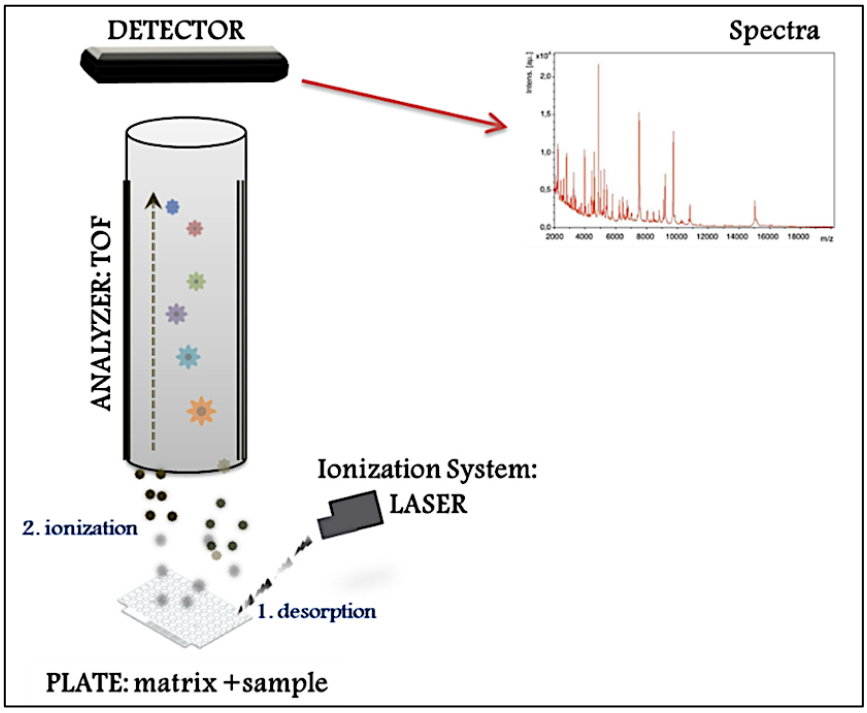

Figure 13: MALDI-TOF MS schematic representation (Torres-Sangiao et al., 2021)

4. Antibiotic susceptibility testing

4.1. Disc diffusion

The disc diffusion method was performed according to the CLSI guidelines (CLSI, 2020). Briefly, a 0.5 McFarland bacterial suspension of each tested isolate was prepared in sterile distilled water then swabbed on the entire surface of a Mueller Hinton agar plate within 15 minutes of its preparation. Afterwards, 16 antibiotic discs were placed on the inoculated plate (Table 4).

The inhibition diameters around each disc were measured after a 24 h incubation at 37 °C, then interpreted according to CLSI breakpoints (CLSI, 2020).

Table 4: List of antibiotics used in the disc diffusion method

Antibiotic family	Antibiotic agent	Abbreviation	Disc content
β-lactams	Ampicillin	AM	10 µg
	Amoxicillin-clavulanic acid	AMC	20 + 10 µg
	Cefotaxime	CTX	30 µg

	Ceftazidime	CAZ	30 µg
	Cefoxitin	FOX	30 µg
	Cefepime*	FEP	30 µg
	Aztreonam	ATM	30 µg
	Ertapenem	ETP	10 µg
	Imipenem	IMP	10 µg
Fluoroquinolones	Ciprofloxacin	CIP	5 µg
	Ofloxacin	OFX	5 µg
	Nalidixic acid**	NA	30 µg
Tetracyclines	Tetracycline	TE	30 µg
	Doxycycline	DO	30 µg
Aminoglycosides	Gentamicin	CN	10 µg
	Amikacin	AK	30 µg
Sulfonamides	Trimethoprim-sulfamethoxazole	SXT	25 µg

***** Cefepime was only tested for cefotaxime-resistant *E. coli*.

****** Nalidixic acid was only tested for colistin-resistant *E. coli*.

4.2.Broth micro-dilution

Colistin minimum inhibitory concentration (MIC) was determined using the broth microdilution method on sterile microtiter trays as previously described (NCDC, 2020). The microdilution tray is composed of 96 round bottom wells, which are coded horizontally from 1 to 12 and vertically from A to B (Figure 14).

4.2.1. Preparation of cation adjusted Mueller Hinton broth (Ca-MHB):

The cation adjusted Mueller Hinton broth was prepared by adding Mg^{2+} and Ca^{2+} cations to the MHB at 1 µg/mL and 2 µg/mL concentrations, respectively. After preparation, 50 µL of Ca-MHB was dispensed in wells 1 to 10 from the left, 75 µL in the 11^{th} well and 100 µL in the 12^{th}.

4.2.2. Preparation of bacterial suspension and dilution:

A bacterial suspension of each tested strain was prepared and adjusted to 0.5 McFarland. Later, 10 µL of each suspension was mixed with 740 µL of Ca-MHB to obtain a

1/75 concentration. Lastly, 25 µL of the final solution was inoculated in each of the wells from 1 to 11.

4.2.3. Preparation of colistin working solution and dilutions:

The working solution was prepared by adding 64 µL volume of the colistin stock solution (1 mg/mL) to 936 µl of Ca-MHB, to achieve a concentration of 64 µg/ml.

Afterward, a 2-fold serial dilution was realized in 9 microtubes by adding 500 µL of the previous colistin solution to 500 µL of Ca-MHB to get 32 µg/mL concentration, then 16 µg/ml, 8 µg/ml, and so on till 0,125 µg/ml.

4.2.4. Inoculation of the microtiter plate:

The final volume in each well was‡ 100 µL. Each well from 1 to 10 is inoculated with:

- 50 µL of Ca-MHB.
- 25 µL of a 1:75 dilution of the tested isolate.
- 25 µL of serial dilutions of the colistin, which are thus further diluted at 1/4 in the final volume of 100 µL, giving final concentrations of 16 µg/mL to 0,03 µg/mL from the 1st to the 10th well respectively.

The 11th and the 12th wells correspond to positive and negative controls, respectively:

- 25 µL of the bacterial dilution is added to 75 µL of Ca-MHB in the 11th well to verify the bacterial growth.
- The last well contains only 100 µL of Ca-MHB to confirm its sterility.

4.2.5. MIC determination:

After incubation at 37°C for 24h, the minimum inhibitory concentration of colistin is determined visually and corresponds to the lowest concentration inhibiting the bacterial growth entirely (absence of turbidity). The obtained MICs were interpreted according to the CLSI breakpoints (CLSI, 2020).

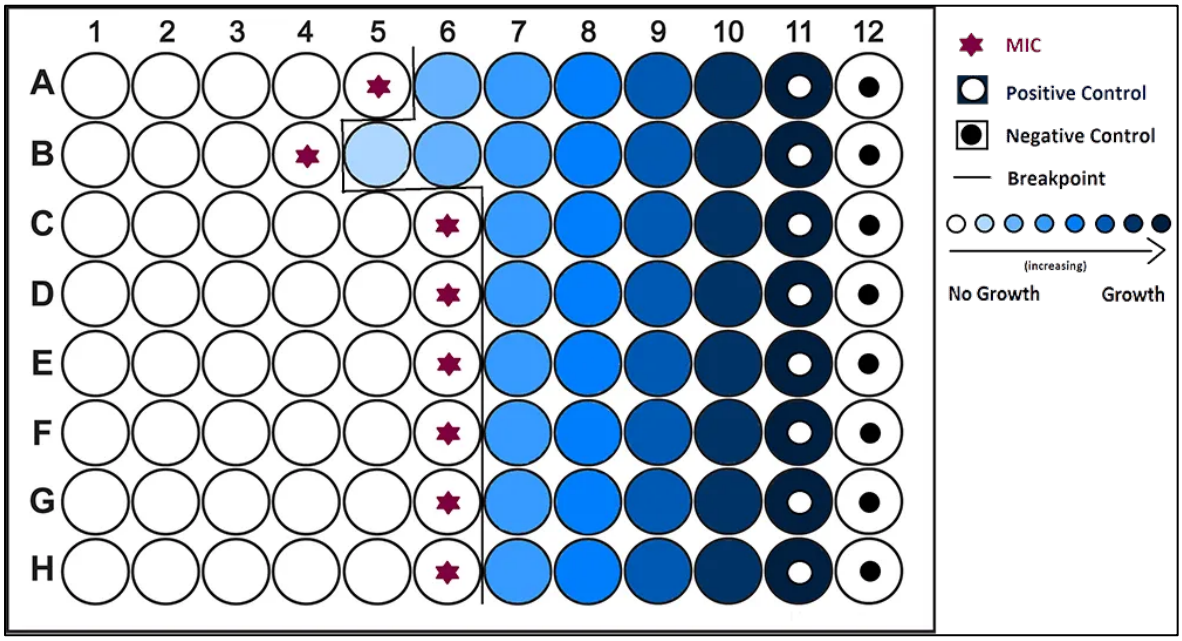

Figure 14: Example of minimum inhibitory concentration on a 96-well microtiter plate. (https://emerypharma.com/blog/minimum-inhibitory-concentration-mic/)

5. Phenotypic characterization of resistance mechanisms

5.1. ESBL detection

The phenotypic detection of ESBL enzymes production was performed concurrently with the double disc synergy test (DDST) and the combination disc test (CDT) for isolates screened for their resistance to cefotaxime as previously described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017).

5.1.1. Double disc synergy test (DDST):

This test was performed on MH plate inoculated with a 0,5 McFarland suspension of the tested isolate, by placing two cephalosporin discs, cefotaxime and ceftazidime, as well as an aztreonam disc next to an amoxicillin-clavulanate disc at a distance of 25 mm center to center. The production of ESBL enzyme was determined by the extension of the inhibition zone around one, two, or all the three discs in the direction of the amoxicillin-clavulanate disc. The observed form is called “keyhole” (EUCAST, 2017) (Figure 15).

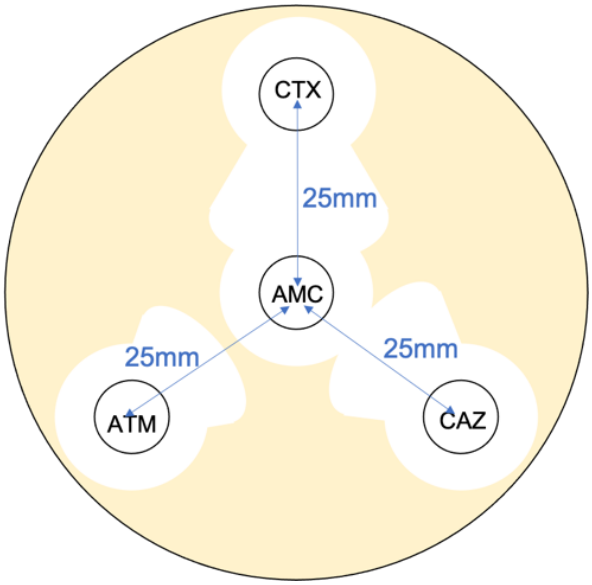

Figure 15: Schematic representation of the double disc synergy test.

5.1.2. Combination disc test:

An amoxicillin-clavulanate disc was placed on an inoculated MH plate then replaced with a cefotaxime disc after 1 hour of diffusion. After incubation at 37°C for 24h, the positive result was revealed by the augmentation of the inhibition diameter around the cefotaxime disc combined to clavulanic acid by ≥ 5 mm compared to the diameter around the cefotaxime alone (EUCAST, 2017) (Figure 16).

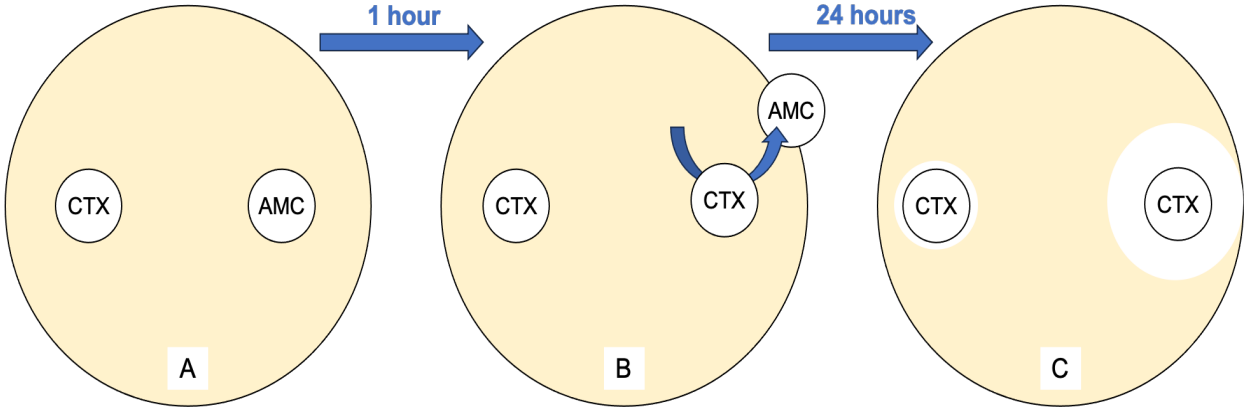

Figure 16: Schematic representation of the combination disc test

(**A:** Placement of cefotaxime and amoxicillin-clavulanate discs on MH agar, **B:** Replacement of the amoxicillin-clavulanate disc with cefotaxime disc after 1 hour, **C:** Increase of the inhibition zone around the cefotaxime disc combined to amoxicillin-clavulanate compared to cefotaxime alone ≥ 5 mm)

5.2. Carbapenemase detection

The production of carbapenemase enzymes was detected using EDTA test for all ertapenem- and/or imipenem- resistant isolates. This test is based on the combination of a carbapenem disc with ethylenediaminetetraacetic acid (EDTA) which inhibits metallo-β-lactamase enzymes (EUCAST, 2017). Shortly, 2 discs of imipenem were placed on MH plate inoculated with a 0,5 McFarland bacterial suspension, then one of them was soaked with 10 µL of EDTA solution (0,5mM) (Yong et al., 2002). The result was revealed after 24h incubation by the increasing of the inhibition zone of the imipenem disc combined to EDTA by 5 mm in comparison with the disc alone (Figure 17).

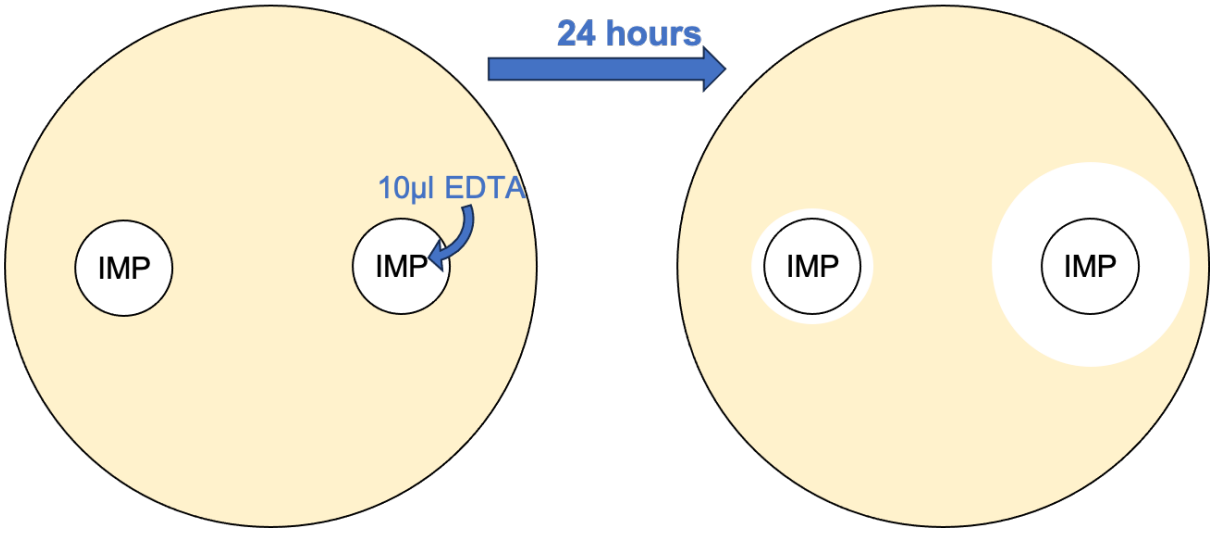

Figure 17: Schematic representation of the EDTA test.

6. Molecular characterization of resistant isolates

6.1. DNA extraction and quantification

The extraction of bacterial DNA was realized following the boiling method with slight modifications (Nguyen et al., 2016). Briefly, 3 to 5 representative bacterial colonies of fresh culture were suspended in 150 µL of sterile Tris EDTA buffer (pH=8). After homogenization, the suspension was boiled for 10 minutes at 95°C, then centrifuged for 10 minutes at 12000 rpm. Later, the supernatant was recovered and analyzed with a NanoDrop™ 8000 spectrometer (Thermo Fisher Scientific, USA) to verify the DNA purity and concentration.

6.2. Polymerase chain reaction (PCR)

The investigation of antibiotic resistance determinants was conducted with standard PCR at the Center of Biotechnology research (CRBT), Constantine, Algeria, the Veterinary Research Institute (IRVT), Tunis, Tunisia. A total of 10 resistance genes were inspected in all 3GC-R *E. coli* isolates: *bla*CTX-M, *bla*NDM-1, *bla*OXA-48, blaVIM, *bla*CMY, *bla*DHA, *aac(6')-ib*, *tet*A, *tet*B and tetC, in addition to class 1 and 2 integron genes *intI1* and *intI2* that were investigated with multiplex PCR. On the other hand, *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5* genes were investigated for CL-R strains.

The PCR reactions were carried out in a 25 µL final volume containing 2,5 µL of 10x buffer, 0,5 µL $MgCl_2$ 50mM, 0,5 µL dNTPs 10mM, 0,5 µL of each primer, 0,25 µL Taq polymerase (5U/µL), and 2 µL of the bacterial DNA. Nuclease-free water was added to reach final volume. The amplification was performed in a Veriti™ thermal cycler (Applied Biosystems, Thermo Fisher Scientific, USA) under the conditions represented in table 5. Finally, the PCR products were revealed by electrophoresis migration at 120 V on a 1,5% agarose gel stained with SYBR™ Safe gel stain (Invitrogen, Thermo Fisher Scientific, USA). The gel was later visualized using a Gel Doc™ XR+ molecular imager with Image Lab™ software (Bio-Rad Laboratories, USA).

The bla_{OXA-48} positive PCR products were further sequenced using a 3500 XL Genetic Analyzer (Thermo Fisher Scientific, USA). The obtained sequences were confirmed with NCBI BLAST program (http://www.ncbi.nlm.nih.gov/BLAST).

Table 5 : Investigated genes and PCR conditions

Genes	Sequences	PCR conditions	Size (pb)	References
bla_{CTX-M}	F-TTTGCGATGTGCAGTACCAGTAA R-CGATATCGTTGGTGGTGCCATA	94°C/10 min, 30 cycles (94°C/40s, 59°C/40s, 72°C/1 min), 72°C/7 min	500	(Kiiru et al., 2012)
bla_{CMY}	F-ATGATGAAAAAATCGTTATGC R-TTGCAGCTTTTCAAGAATGCGC	95°C/15 min, 30 cycles (94°C/30s, 50°C/30s, 72°C/2 min), 72°C/10 min	1200	(Kiiru et al., 2012)

***bla*$_{DHA}$**	F-TGATGGCACAGCAGGATATTC R-GCTTTGACTCTTTCGGTATTCG	94°C/10 min; 30 cycles (94°C/40 s, 60°C/40s, 72°C/1 min), 72°C/7 min	997	(Dallenne et al., 2010)
***bla*$_{NDM-1}$**	F-GCTTTGGCGATCTGGTTTTC R-CGGAATGGCTCATCACGATC	94°C/10 min, 36 cycles (94°C/30s, 52°C/40s, 72°C/50 s), 72°C/5 min	621	(Poirel et al., 2011)
***bla*$_{OXA-48}$**	F-TTGGTGGCATCGATTATCGG R- GAGCACTTCTTTTGTGATGGC	95°C/15 min, 30 cycles (94°C/30s, 54°C/30s, 72°C/2min), 72°C/10 min	744	(Mellouk et al., 2017)
bla$_{VIM}$	F-GATGGTGTTTGGTCGCATA R-CGAATGCGCAGCACCAG	94°C/10 min, 30 cycles (94°C/40s, 55°C/40s, 72°C/1 min), 72°C/7 min	390	(Dallenne et al., 2010)
***tet*A**	F-GTAATTCTGAGCACTGTCGC R-CTGCCTGGACAACATTGCTT	95°C/5 min, 25 cycles (94°C/30s, 62/30s, 72°C 45s), 72°C 7min	937	(Guardabassi et al., 2000)
***tet*B**	F-CTCAGTATTCCAGCCTTTG R-CTAAGCACTTGTCTCCTGTT	95°C/5 min, 25 cycles (95°C/30s, 57°C/30s 72°C 20s), 72°C 7 min	416	(Guardabassi et al., 2000)
***tet*C**	F-TCTAACAATGCGCTCATCGT R-GGTTGAAGGCTCTCAAGGGC	95°C/5 min, 25 cycles (94°C/30s, 62/30s, 72°C 45s), 72°C 7min	570	(Guardabassi et al., 2000)
aac(6')-ib	F-TTGCGATGCTCTATGAGTGGCTA R-CTCGAATGCCTGGCGTGTTT	95°C/5 min, 34 cycles (94°C/45s, 55°C/45s, 72°C/45s), 72°C/7 min	482	(Park et al., 2006)
intI1	F-GGGTCAAGGATCTGGATTTCG R-ACATGGGTGTAAATCATCGTC	95°C/5 min, 30 cycles (94°C/30s, 62°C/30s, 72°C/1 min), 72°C/5 min	483	(Mazel et al., 2000)
intI2	F-CACGGATATGCGACAAAAAGGT R-GTAGCAAACGAGTGACGAAATG		788	

qacΔE-sul1	F-GGCTGGCTTTTTCTTGTTATCG R-GCGAGGGTTTCCGAGAAGGTG	94°C/5 min, 30 cycles (94°C/30s, 63°C/30s, 72°C/1 min), 72°C/8 min	1125	(Sáenz et al., 2004)
mcr-1	F-AGTCCGTTTGTTCTTGTGGC R-AGATCCTTGGTCTCGGCTTG	94°C/15 min, 25 cycles (94°C/30s, 58°C/90s 72°C/60s), 72°C/10 min.	320	(Rebelo et al., 2018)
mcr-2	F- CAAGTGTGTTGGTCGCAGTT R- TCTAGCCCGACAAGCATACC	94°C/15 min, 25 cycles (94°C/30s, 58°C/90s 72°C/60s), 72°C/10 min.	715	
mcr-3	F-AAATAAAAATTGTTCCGCTTATG R-AATGGAGATCCCCGTTTTT	94°C/15 min, 25 cycles (94°C/30s, 58°C/90s 72°C/60s), 72°C/10 min.	929	
mcr-4	F-TCACTTTCATCACTGCGTTG R-TTGGTCCATGACTACCAATG	94°C/15 min, 25 cycles (94°C/30s, 58°C/90s 72°C/60s), 72°C/10 min.	1116	
mcr-5	F-ATGCGGTTGTCTGCATTTATC R- TCATTGTGGTTGTCCTTTTCTG	94°C/15 min, 25 cycles (94°C/30s, 58°C/90s 72°C/60s), 72°C/10 min.	1644	

6.3.Whole-genome sequencing

Whole genome sequencing was conducted at the Limoges University Hospital in France and was exclusively performed on *mcr*-positive CL-R strains in order to characterize their genetic profiles, identify their resistance determinants and investigate their genetic relatedness.

6.3.1. DNA extraction and libraries preparation:

Genomic DNA was extracted using the SaMag automaton with the Bacterial DNA Extraction kit (Sacace Biotechnologies). The DNA purity and quantity were verified with a NanoDrop^{TM} 8000 spectrometer (Thermo Fisher Scientific, USA). Afterwards, the DNA was utilized to create libraries using the Ion Xpress Plus Fragment Library kit on the AB Library Builder system (Thermo Fisher Scientific) following the manufacturer's protocol.

6.3.2. Sequencing:

The sequencing was conducted on the Ion Torrent Genestudio™ S5 platform (Thermo Fisher Scientific) according to the manufacturer's guidelines. The quality of Raw-reads was evaluated using FastQC v0.11.9 and MultiQC v1.21.

6.3.3. Reading alignment and genome assembly:

Reads were aligned against an *E. coli* reference genome (GenBank accession number CP042470) using BWA v0.7.17-r1188 and the read count was assessed with Samtools v1.15.1. All strains achieved a minimum depth of 80x. Finally, the read assembly was conducted using SPADES v3.13.0, providing a high-quality draft genome for subsequent analyses.

6.3.4. Multi-locus sequence typing (MLST):

The MLST was conducted on the pubMLST database to determine sequence types (STs) of the *mcr*-positive isolates and assess their clonal relatedness. In parallel, core genome Single Nucleotide Polymorphisms (SNPs) were identified with Snippy v4.4.5 using the *E. coli* CP042470 strain as a reference in order to achieve higher-resolution phylogenetic comparison between the strains. Subsequently, a phylogenetic tree was built in order to analyze strain relationships according to SNP comparisons using FastTree v2.1.10 and visualized on FigTree v1.4.4 software.

6.3.5. In silico analysis of resistance genes and plasmids:

The assembled genome sequences were analyzed in order to screen acquired resistance genes and determine plasmid replicon types using RefFinder 4.1 and PlasmidFinder 2.1 respectively.

Results

1. Overall detection of resistant *Escherichia coli* isolates:

Overall, 112 *Escherichia coli* strains were isolated from 919 samples (12 %), including 58 third generation cephalosporin-resistant (3GC-R) strains and 54 colistin-resistant (CL-R).

The isolates were detected among 21 of the 38 investigated farms (66 %), of which 5 harbored CL-R *E. coli* (13 %) and 20 were positive for 3GC-R *E. coli* (53 %). The co-detection of both 3GC resistance and colistin resistance was observed in 4 farms (11 %) (Figure 18).

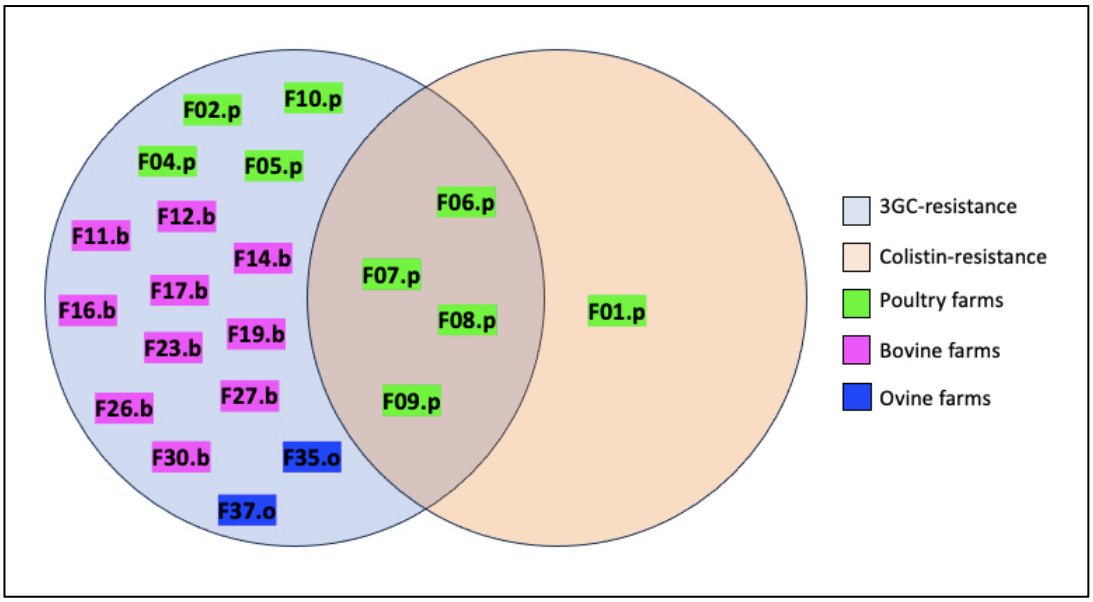

Figure 18: Venn Diagram of colistin- and 3GC-resistance in the farms

2. Detection of 3GC-R *E. coli*:

2.1. Detection of 3GC-R *E. coli* in poultry farms:

Overall, 31 3GC-R *E. coli* isolates were detected across 8/10 poultry farms (80 %) (Figure 19-B). The positive farms were distributed among 4 regions: Medjez Ammar (F02.p, F08.p, and F09.p), Guelaât Bou Sbaâ (F04.p and F05.p), Bendjerrah (F06.p and F07.p), and Bouati Mahmoud (F10.p).

2.2. Detection of 3GC-R *E. coli* in bovine and ovine farms:

A total of 27 3GC-R isolates were isolated from the sampled ruminant farms, including 25 isolates from 10 bovine farms and 2 from 2 ovine farms. Cattle farms exhibited a higher prevalence of 48 % (10/21) (Figure 19-C), compared to sheep farms 29 % (2/7) (Figure 19-D).

The bovine farms were located across three different regions: Heliopolis (F11.b, F12.b, F14.b, F16.b and F17.b), Bouati Mahmoud (F19.b, F26.b, F27.b and F30.b), and Hammam Debagh (F23.b), while the 2 ovine farms (F35.o and F37.o) were both situated in the Bouati Mahmoud region.

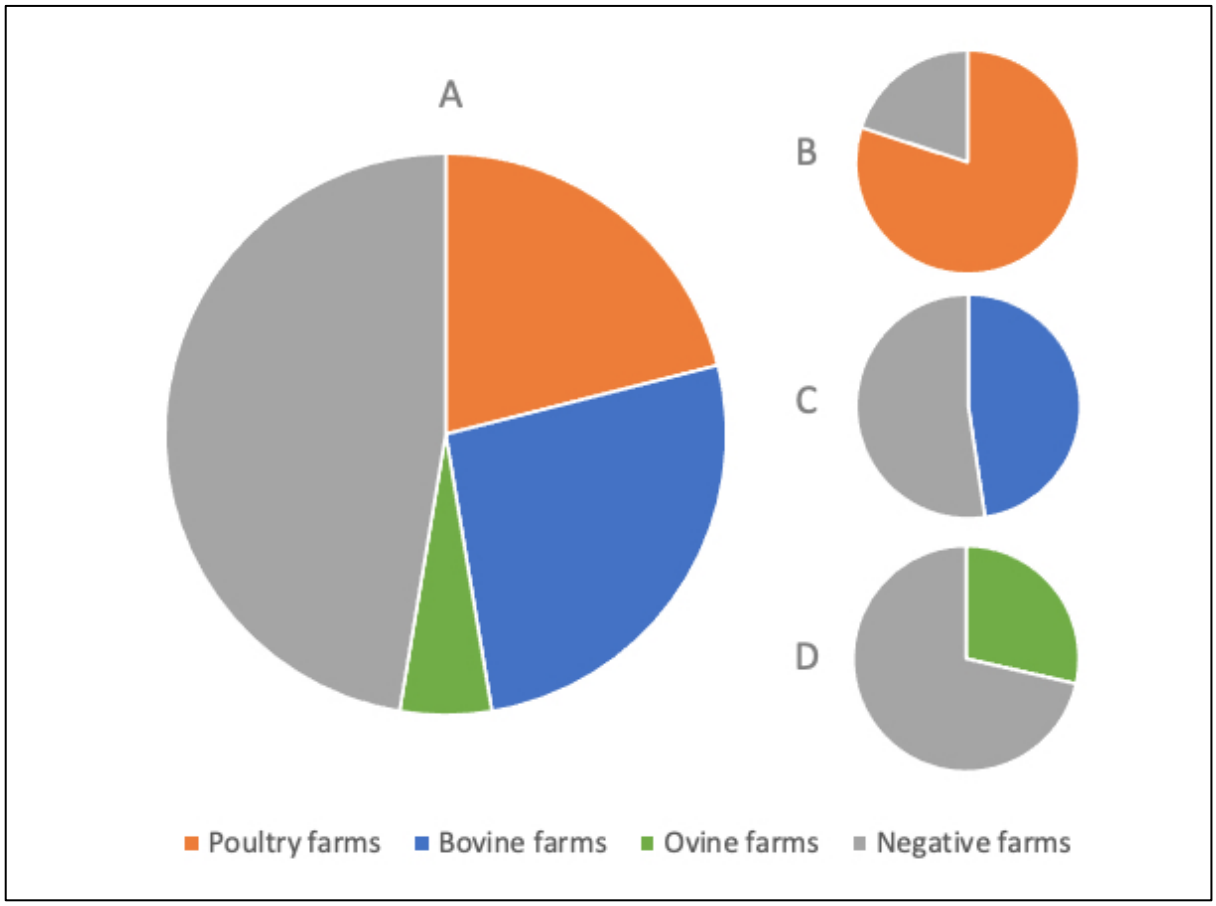

Figure 19: Prevalence of 3GC-resistance in the farms (**A:** Overall prevalence; **B:** Prevalence in poultry farms; **C:** Prevalence in bovine farms; **D:** Prevalence in ovine farms)

3. Detection of CL-R *E. coli* (Longitudinal detection of CL-R isolates in poultry farms)

CL-R *E. coli* isolates were exclusively detected in poultry farms, while they were absent in bovine and ovine farms. Therefore, this section focuses on the longitudinal occurrence of CL-R isolates in poultry across successive production cycles

During the overall sampling period, a total of 54 CL-R *E. coli* isolates were identified among 5 of the 10 sampled poultry farms (50 %), including F01.p in the Heliopolis region, F06.p and F07.p in the Bendjerrah region, and F08.p and F09.p in the Medjez Ammar region.

The isolates were detected across multiple sampling campaigns and in different sample types (Figures 20).

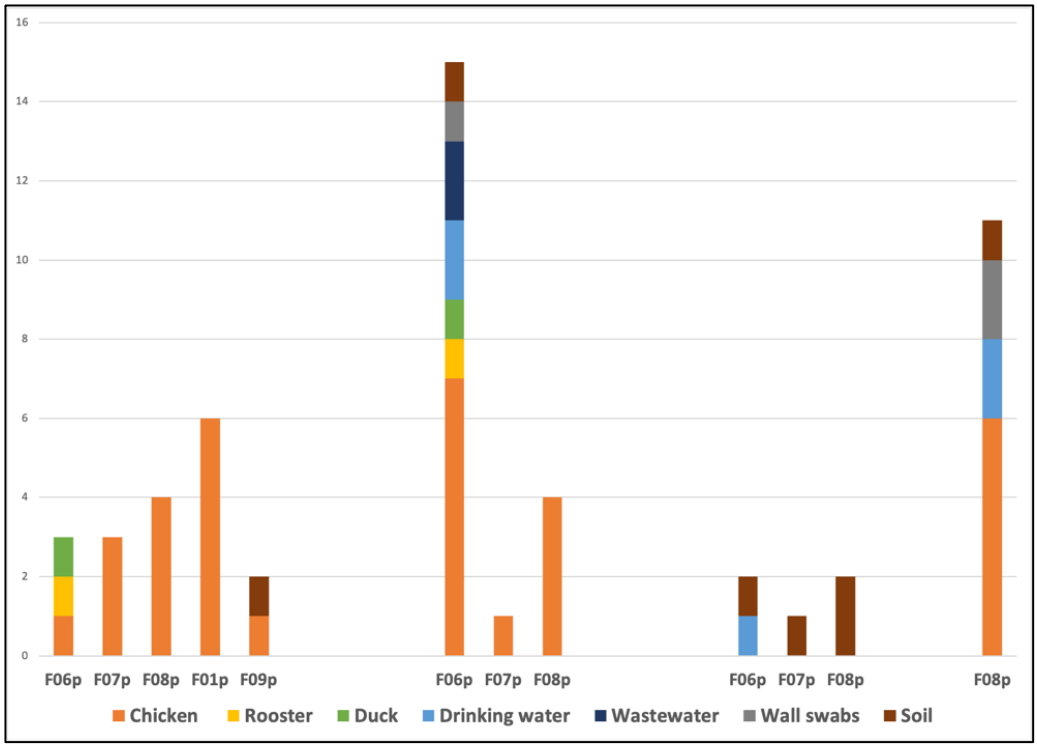

Figure 20: Longitudinal detection of CL-R isolates in poultry farms

3.1. First sampling campaign:

In this campaign 18 CL-R *E. coli* isolates were detected:

- **Farm F01.p:** 6 isolates in chicken fecal samples.
- **Farm F06.p:** 3 isolates in chicken, rooster, and duck samples.
- **Farm F07.p:** 3 isolates in chicken samples.
- **Farm F08.p:** 4 isolates in chicken samples.
- **Farm F09.p:** 2 isolates in chicken and soil samples.

3.2. Second sampling campaign:

From the second sampling time and beyond, only farms F06.p, F07.p and F08.p were sampled owing to their accessibility. All three farms were positive in the second campaign with a total of 20 isolates detected:

- **Farm F06.p:** 15 isolates in chicken (n=07), duck (n=01), and rooster (n=01) fecal samples, soil (n=01), wastewater (n=02), drinking water (n=02), and wall swab (n=01)
- **Farm F07.p:** one isolate in a chicken sample.
- **Farm F08.p:** 4 isolates in chicken samples.

3.3. Third sampling campaign:

4 CL-R isolates were detected in this campaign, only from environmental samples:

- **Farm F06.p:** Two isolates in soil (n=01) and drinking water (n=01).
- **Farm F07.p:** one isolate in a soil sample.
- **Farm F08.p:** Two isolates in soil samples.

3.4. Fourth sampling campaign:

Only farm F08.p was sampled in this campaign since the third sampling was conducted in the absence of animals. In this sampling, 11 isolates were detected in chicken (n=06), soil (n=01), drinking water (n=02), and wall swabs (n=02).

4. Data collected from poultry farms F06.p, F07.p and F08.p

The data on antibiotic usage and disinfection protocols were collected in farms F06.p, F07.p and F08.p from veterinarians and farmers in order to evaluate their impact on the maintenance of colistin-resistant strains in farms over time (Table 6).

4.1.Antibiotic usage

Colistin was used in the three investigated farms for different purposes. It was employed in farms F06.p and F07.p for all the investigated flocks at the age of 28 days as a prophylactic treatment in combination with doxycycline. Whereas in farm F08.p, a colistin and enrofloxacin association was prescribed for the treatment of respiratory infections in the two first sampled flocks, while no antibiotic was employed in the last flock.

4.2. Disinfection routine

Different protocols of sanitation were followed in the farms between the production cycles. In farm F06.p, quaternary ammonium and glutaraldehyde- based solution was applied

between the first and the second flocks, while another disinfectant consisting of hydrogen peroxide was used between the two other production cycles. Regarding farm F07.p, only chlorine and lime were used between all the cycles. Finally, farm F08.p was cleaned with chlorine between the first and second production cycles and with iodine between the other cycles.

Table 6: Data collected from the poultry farms F06.p, F07.p and F08.p

Farms	Sampling campaign	Date of sampling	Antibiotic usage	Disinfection protocol
F06.p	**1st**	May 2022	Colistin + Doxycycline at day 28 for prophylaxis	No data
	2nd	July 2022	Colistin + Doxycycline at day 28 for prophylaxis	TH5 (quaternary ammonium and glutaraldehyde)
	3rd	September 2022	Colistin + Doxycycline at day 28 for prophylaxis	OXOCID (hydrogen peroxide, peracetic acid and acetic acid)
F07.p	**1st**	May 2022	Colistin + Doxycycline at day 28 for prophylaxis	No data
	2nd	September 2022	Colistin + Doxycycline at day 28 for prophylaxis	Chlorine and lime
	3rd	November 2022	Colistin + Doxycycline at day 28 for prophylaxis	Chlorine and lime
F08.p	**1st**	June 2022	Enrofloxacin in the 1st week for prophylaxis Colistin + enrofloxacin for infection treatment	No data
	2nd	August 2022	Enrofloxacin in the 1st week for prophylaxis Colistin + Enrofloxacin for infection treatment	Chlorine
	3rd	October 2022	No Animals were present	Iodine

	4th	December 2022	Enrofloxacin in the 1st week for prophylaxis	Iodine

5. Variation of the prevalence of positive farms

5.1. Variation by animal species

The prevalence of positive samples and farms varied across animal species (Figure 21). 3GC resistance was significantly higher in bovine samples with a prevalence of 17 % positive samples (25/145), while it was detected at lower rates in poultry and ovine samples with 10 % (31/318) and 1 % (2/140), respectively. However, in terms of farms, a prevalence of 80 % positive farms was found in poultry (8/10), followed by bovine farms and ovine farms with 48 % (10/21) and 29 % (2/7) respectively.

CL-R isolates were only identified among poultry samples, while none was identified in bovine or ovine farms. A total of 35 CL-R *E. coli* strains were detected in 510 poultry samples (7 %). Isolates were present in 50 % of the investigated farms (5/10).

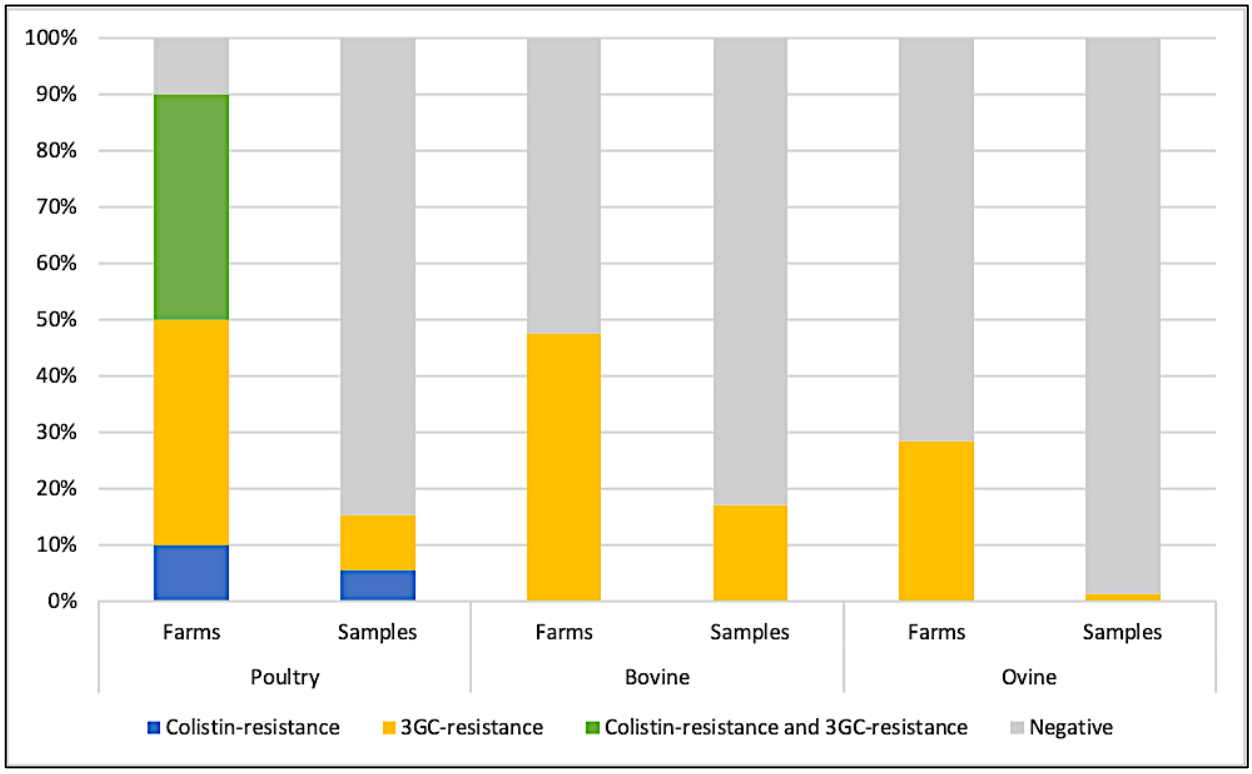

Figure 21: Variation of the resistance prevalence across animal species

5.2. Geographical variation of the prevalence

The prevalence of CL-R and 3GC-R *E. coli* fluctuated over regions (Figure 22). Regarding 3GC-resistance, Bendjerrah and Guelaât Bou Sbaâ regions had a prevalence of 100 % positive farms (2/2 in each region), 3 of the 4 investigated farms in Medjez Ammar were positive (75 %), Heliopolis region comes next with a prevalence of 56 % (5/9), then Bouati Mahmoud with 39 % rate (7/18) and finally Hammam Debagh with a 33 % prevalence (1/3).

On the other hand, colistin resistance was only observed in poultry farms, which were distributed among 3 regions: Bendjerrah with the highest prevalence (100 %) of positive farms (2/2), followed by Medjez Ammar and Heliopolis with respective farm rates of 67 % (2/3) and 50 % (1/2).

Notably, the prevalence of positive farms was higher in urban regions compared to the ones located in rural zones, where 63 % of the farms sampled in urban regions were positive (5/8), against 53 % rate in the rural farms (16/30).

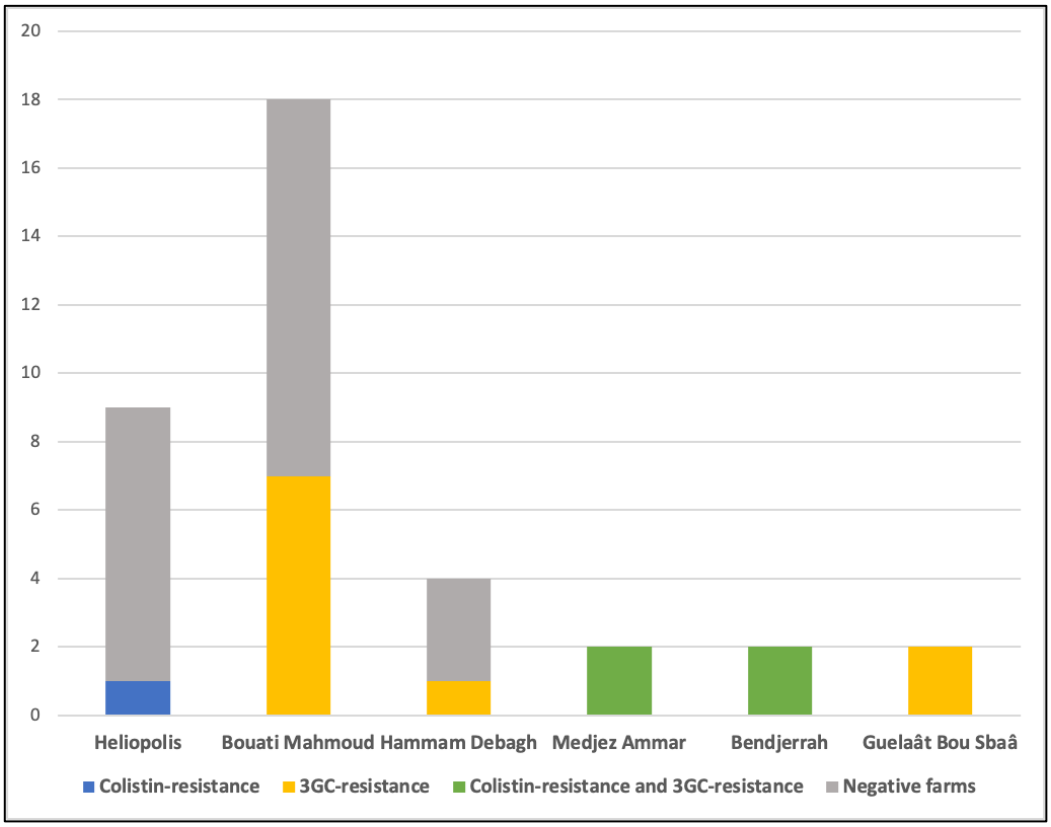

Figure 22: Variation of the resistance prevalence across regions

5.3.Variation by farming system

The overall detection of CL-R and 3GC-R isolates was notably higher in intensive farms 88 % (14/16) compared to the ones managed under semi-extensive system 32 % (7/22) (Figure 23).

Particularly, 3GC resistance was found at a rate of 81 % in intensive farms (13/16) against 3 2% across the semi-extensive farms (7/22), whereas colistin resistance was detected in 31 % of the intensive farms (5/16), and it was absent in all the semi-extensive ones.

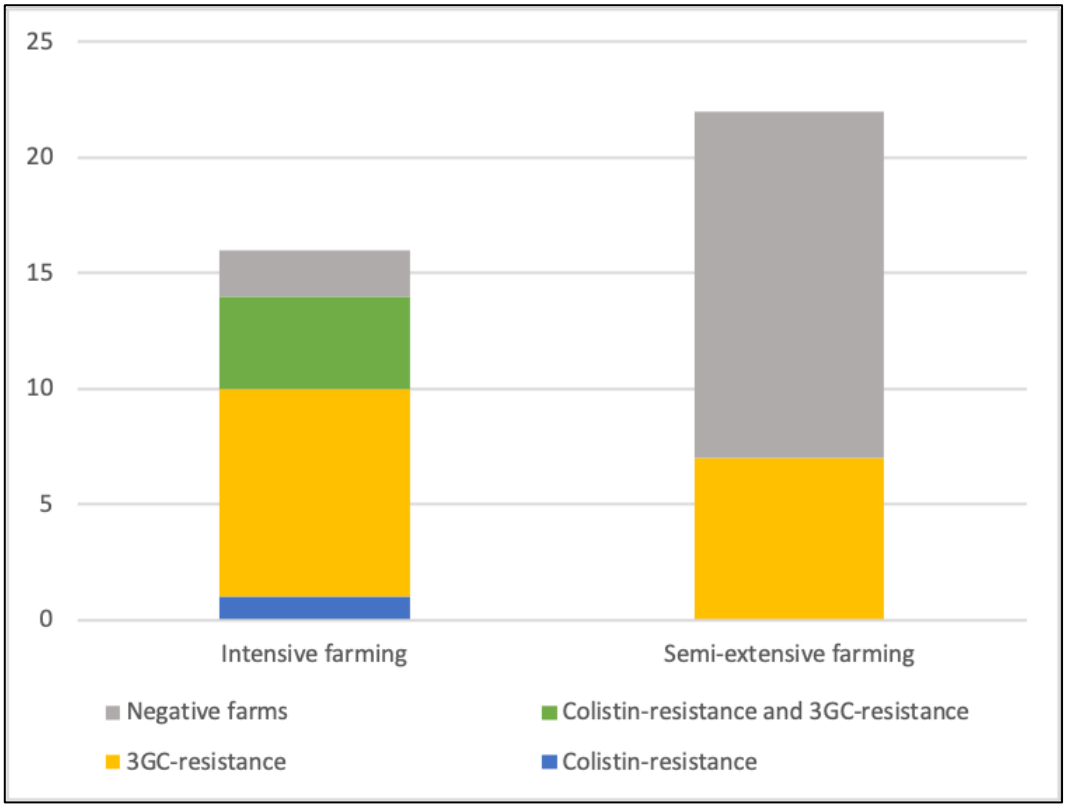

Figure 23: Variation of the resistance prevalence across farming systems

6. Antibiotic susceptibility profiles

6.1.Antibiotic susceptibility profiles of 3GC-R isolates

Overall, 93 % of the 3GC-R *E. coli* strains were multidrug resistant, i.e., resistant to at least one agent in three or more antibiotic classes. Resistance to β-lactams was significantly high with 100 % prevalence to amoxicillin, amoxicillin-clavulanic acid, cefotaxime, cefepime and aztreonam, 50 % and 22 % to ceftazidime and cefoxitin respectively, while resistance rates to ertapenem and imipenem were 24 % and 19 %, respectively. Moreover, the isolates were

highly resistant to fluoroquinolones, tetracyclines and trimethoprim-sulfamethoxazole, with respective rates of 83 % and 86 % against ciprofloxacin and ofloxacin, 90 % and 84 % to tetracycline and doxycycline and 81 % to trimethoprim-sulfamethoxazole. The strains showed low resistance toward aminoglycosides with 17 % and 29 % frequencies to gentamicin and amikacin, respectively. Finally, none of the isolates was resistant to colistin (Figure 24 and Table S4).

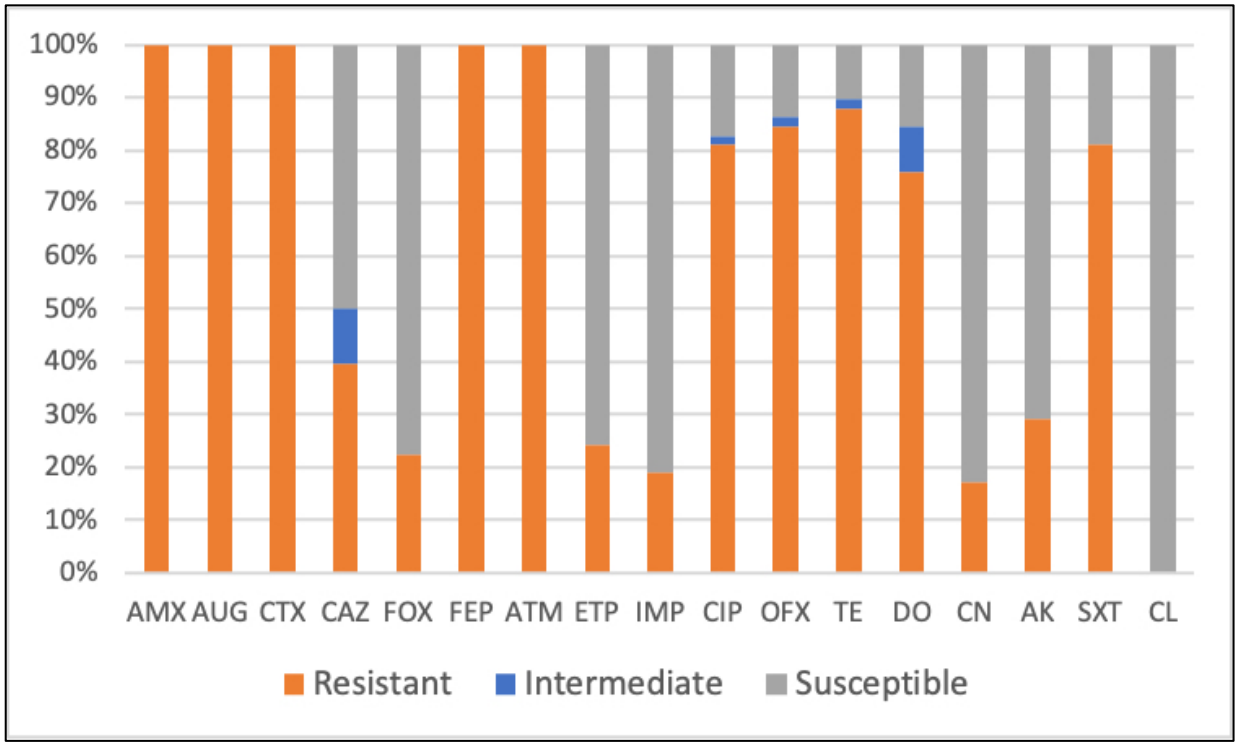

Figure 24: Antibiotic susceptibility profiles of the 3GC-R isolates

6.2. Antibiotic susceptibility profiles of CL-R isolates

All 54 CL-R *E. coli* strains displayed a multidrug resistance phenotype. The colistin MIC varied from 4 µg/mL to ≥16 µg/ml. Isolates displayed high resistance rates against fluoroquinolones and tetracyclines; with resistance rates of 93 %, 87 % and 98% to ciprofloxacin, ofloxacin and nalidixic acid respectively, and 96 % and 85 % to tetracycline and doxycycline respectively, while 57 % were resistant to trimethoprim-sulfamethoxazole. In contrast, resistance to β-lactams was low, apart from amoxicillin and amoxicillin-clavulanic acid to which 67 % of the strains were resistant, whereas all the strains were susceptible to cefotaxime, ceftazidime, cefoxitin, aztreonam, ertapenem and imipenem. Finally, resistance to

aminoglycosides was weak as well with 13 % rate against amikacin while all the strains were susceptible to gentamicin (Figure 25 and Table S5).

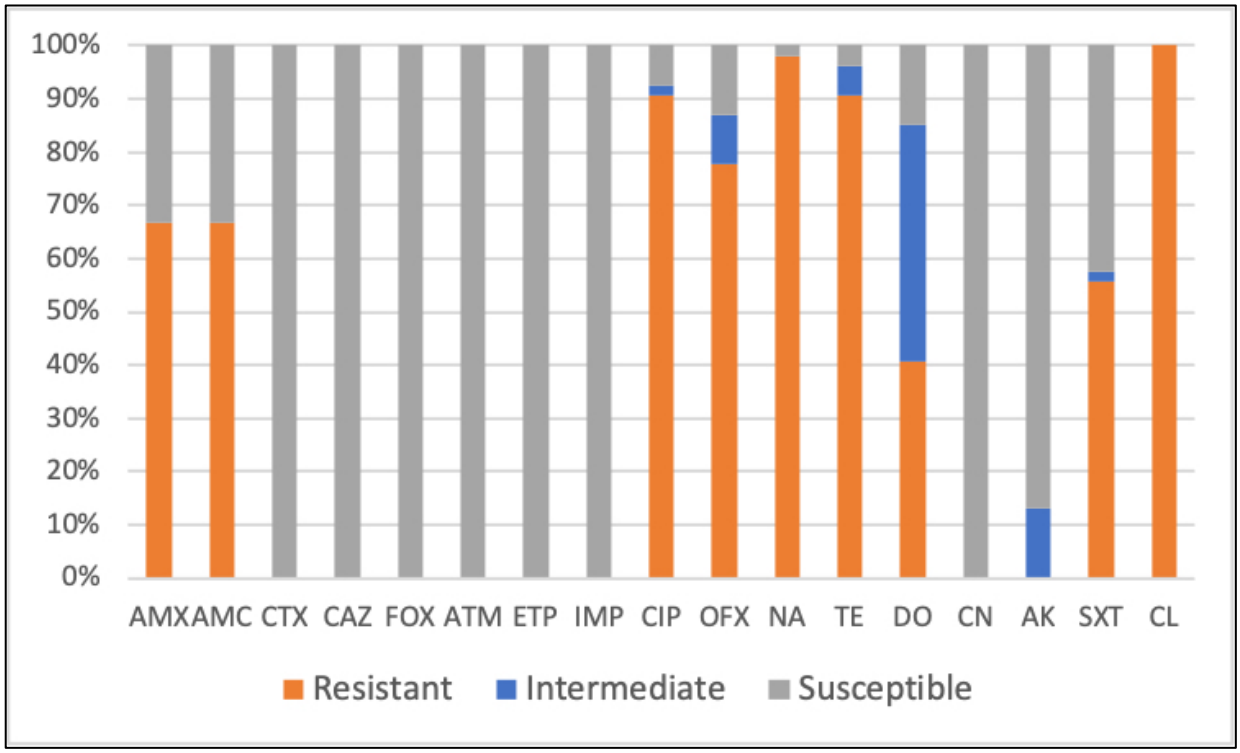

Figure 25: Antibiotic susceptibility profiles of the CL-R isolates

7. Phenotypic characterization of resistance mechanisms

7.1. ESBL detection

According to the phenotypic tests, 42 of the 3GC-R isolates were ESBL producers (72 %), including 29 from poultry samples, 11 bovine, and 2 ovine strains. Particularly, 94 % of the poultry strains (29/31) and 41 % of the bovine strains (11/27) were ESBL producers, while 100 % of the ovine strains were positive (2/2) (Figures 26-28).

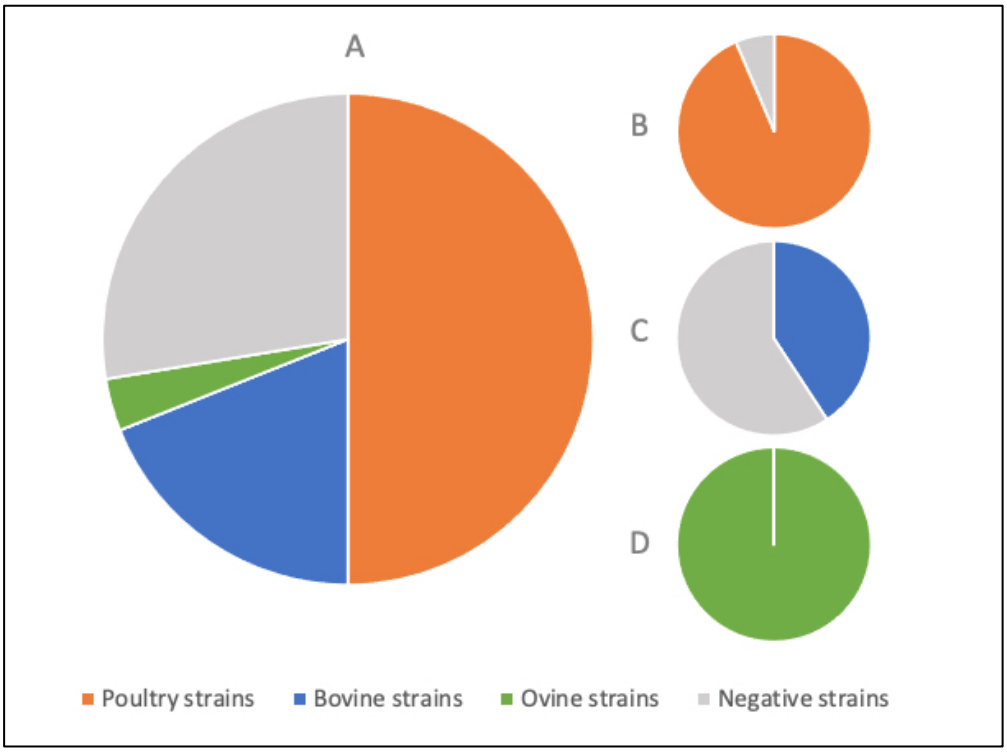

Figure 26: Prevalence of ESBL-producing strains (**A:** Overall prevalence; **B:** Prevalence in poultry strains; **C:** Prevalence in bovine strains; **D:** Prevalence in ovine strains)

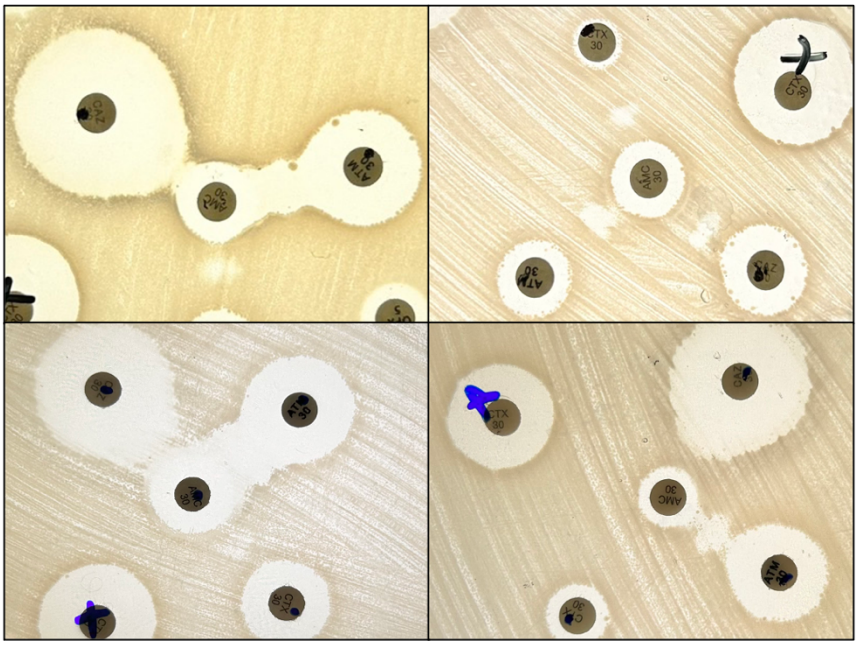

Figure 27: Phenotypic detection of ESBL production with the double disc synergy test

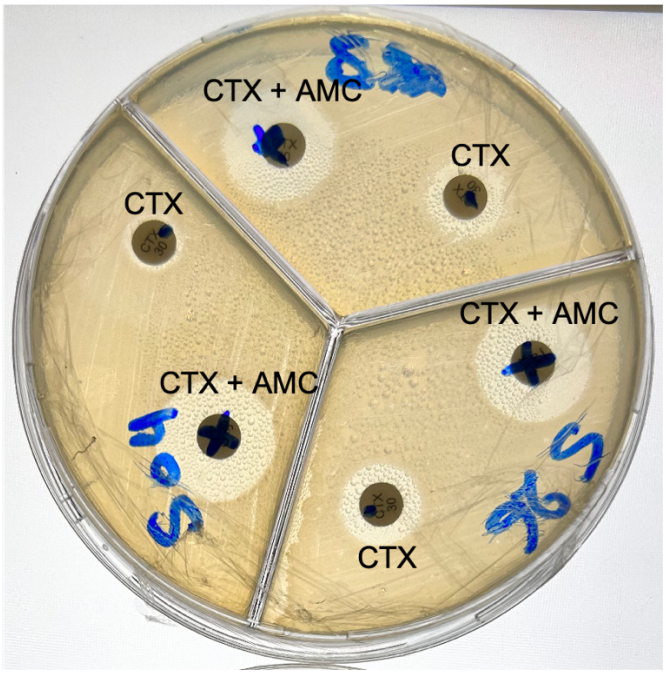

Figure 28: Phenotypic detection of ESBL production with the combination disc test

7.2. Carbapenemase detection

The EDTA test was positive for 10 isolates over the 14 carbapenem-resistant strains tested (71 %), while the overall prevalence of positive strains among 3GC-R isolates was 17 % (10/58). Notably, all strains were isolated from cattle (Figure 29 & 30)

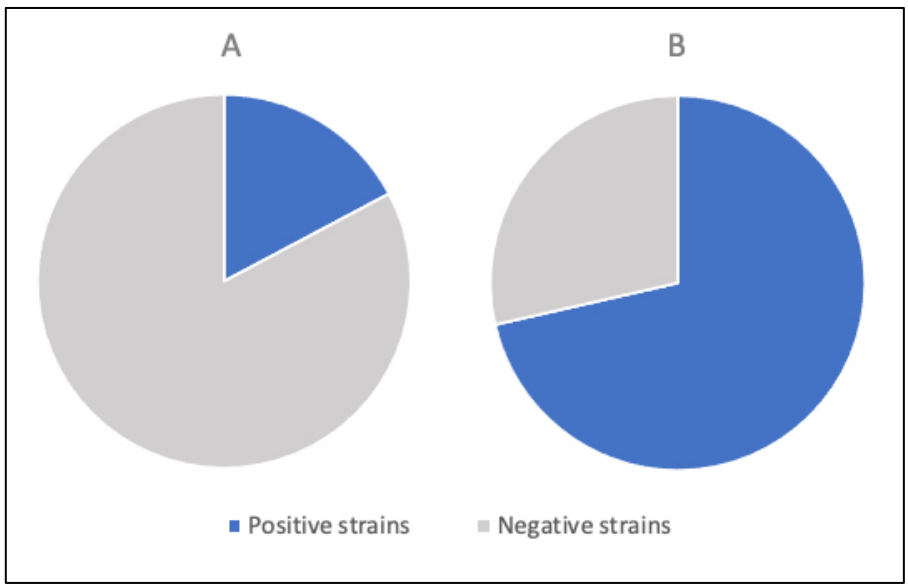

Figure 29: Prevalence of carbapenemase-producing strains (**A:** Overall prevalence in cefotaxime-resistance strains; **B:** Prevalence in carbapenem-resistant strains)

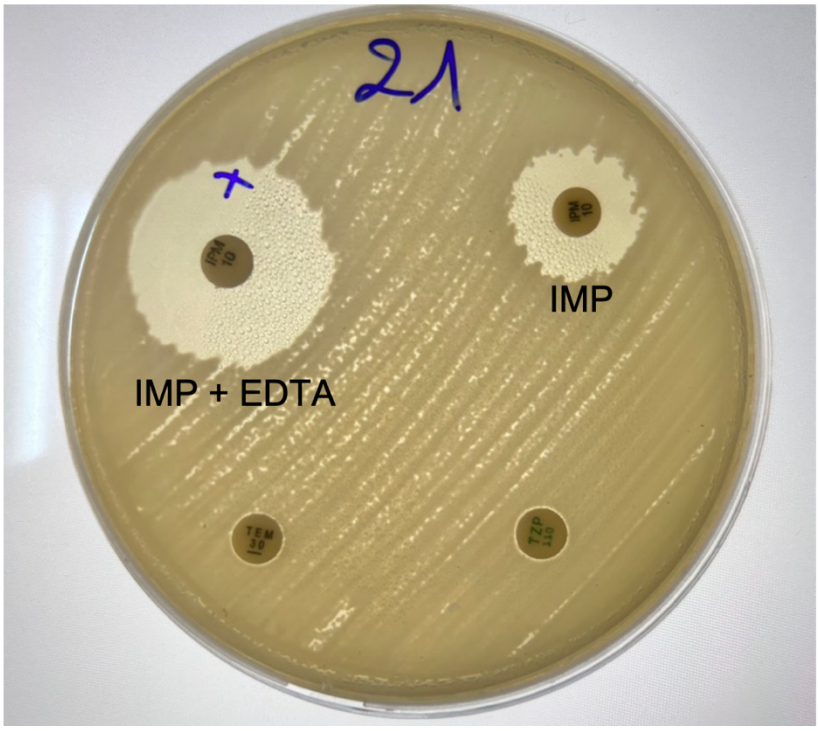

Figure 30: Phenotypic detection of carbapenemases production with the EDTA test

8. Molecular characterization of the *E. coli* isolates

8.1. Molecular characterization of 3GC-R isolates

Several antibiotic-resistance genes were detected in the 3GC-R isolates, with the $bla_{\text{CTX-M}}$ gene being the most prevalent with 87 % rate (51/58). Two carbapenemase genes $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-181}}$ were identified at 17 % (10/58) and 1,7 % (1/58) rates, respectively, while the bla_{VIM} gene was not detected in any of the strains. AmpC genes bla_{CMY} and bla_{DHA} were respectively found in 21 % (12/58) and 1,7 % (1/58) of the isolates. In addition, tetracycline-resistance genes *tet(A)*, *tet(B)* and *tet(C)* were identified at respective frequencies of 17% (10/58), 5 % (3/58) and 3 % (2/58). Moreover, 9 % of the isolates carried the *aac(6')-ib* gene (5/58). Finally, the class 1 integron gene *intI1* was present in 41 % of the strains(24/58), among which 8 amplified the *qacΔE-sul1* segment (14 %), while the *intI2* gene was not found (Figures 31-37 and Table S4).

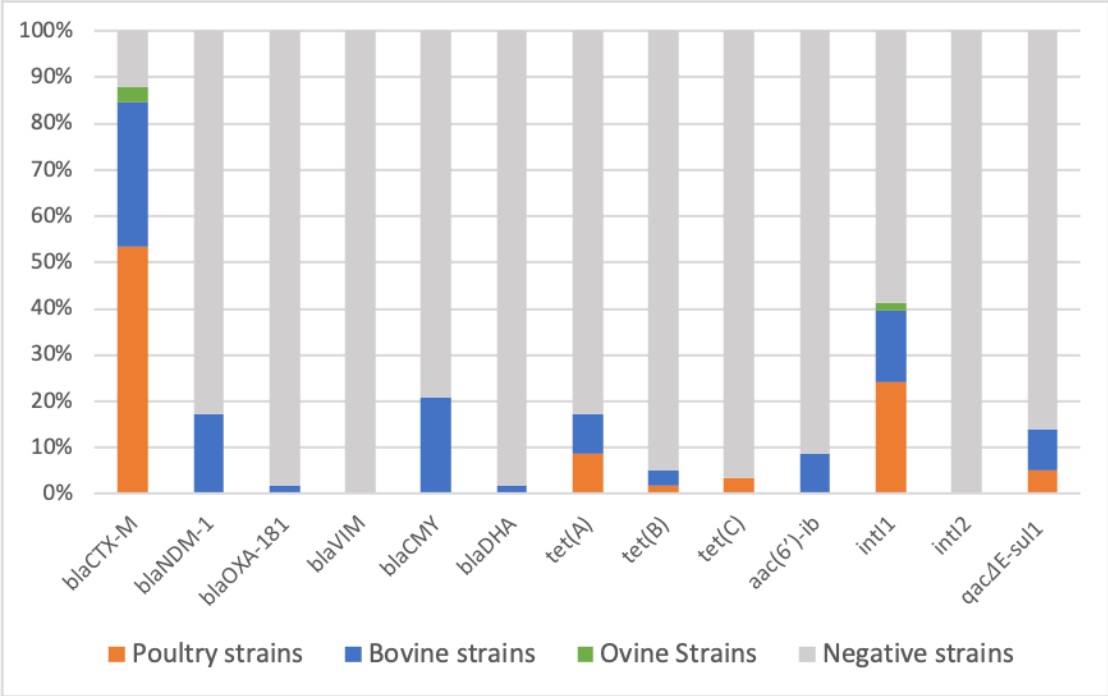

Figure 31: Resistance determinants of the 3GC-R isolates

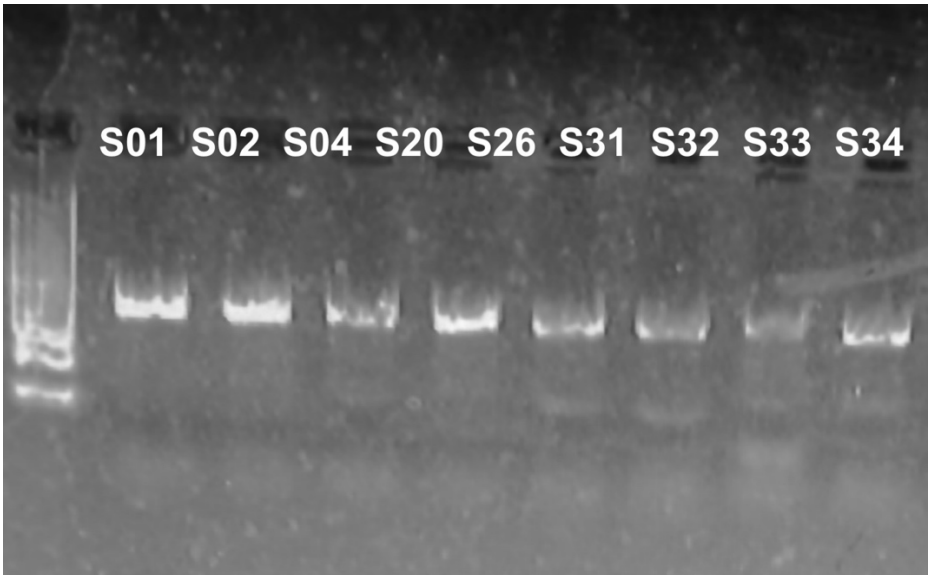

Figure 32: PCR amplification of the *bla*$_{\text{CTX-M}}$ gene

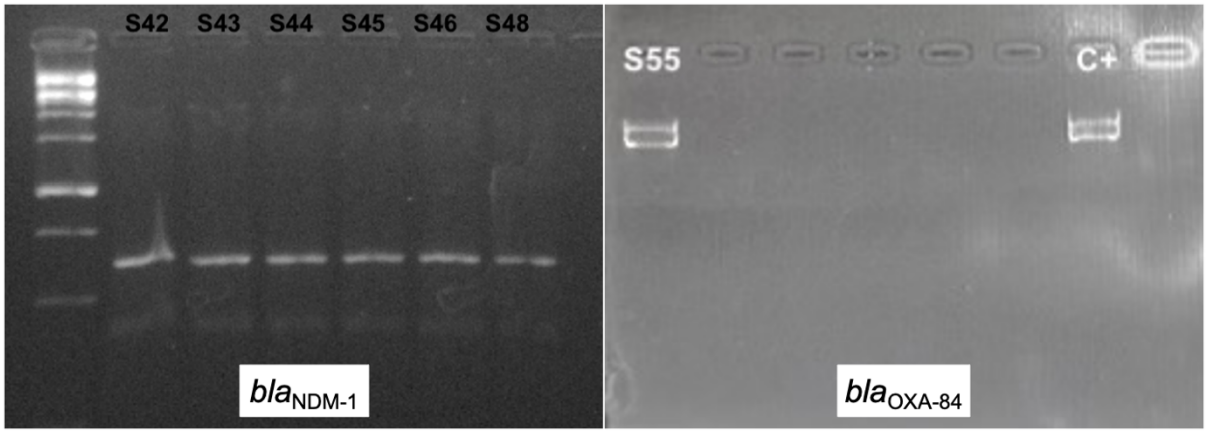

Figure 33: PCR amplification of the bla_{NDM-1} and the bla_{OXA-48} genes

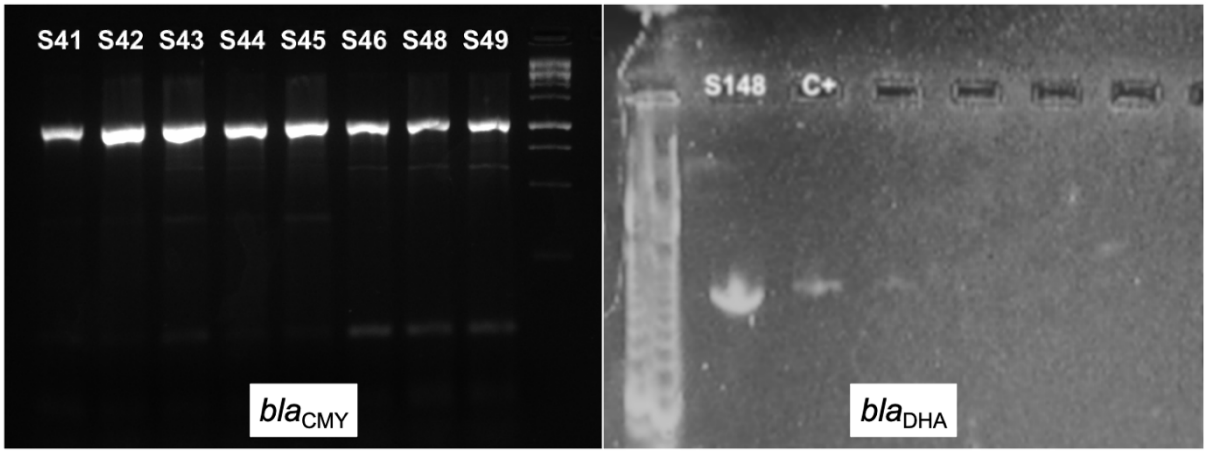

Figure 34: PCR amplification of the bla_{CMY} and the bla_{DHA} genes

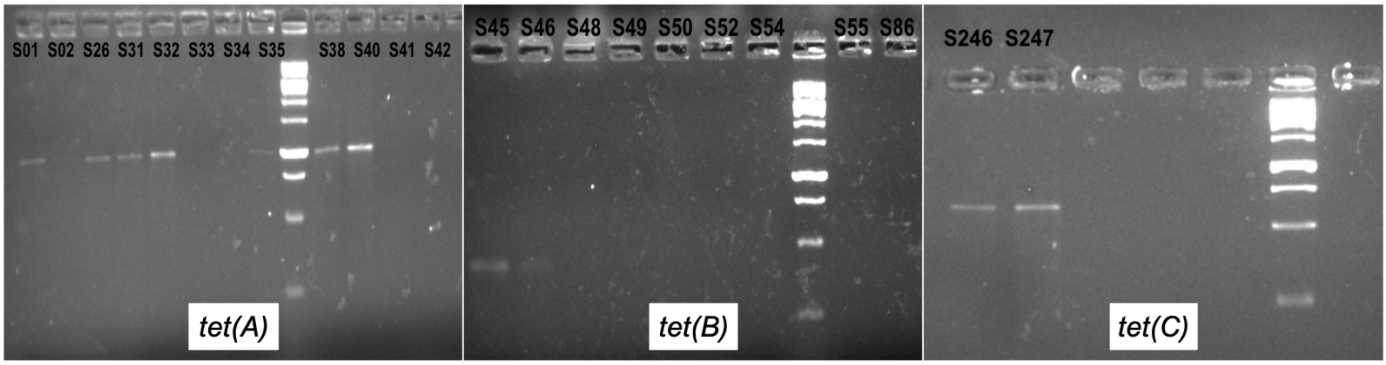

Figure 35: PCR amplification of the *tet(A)*, *tet(B)* and the *tet(C)* genes

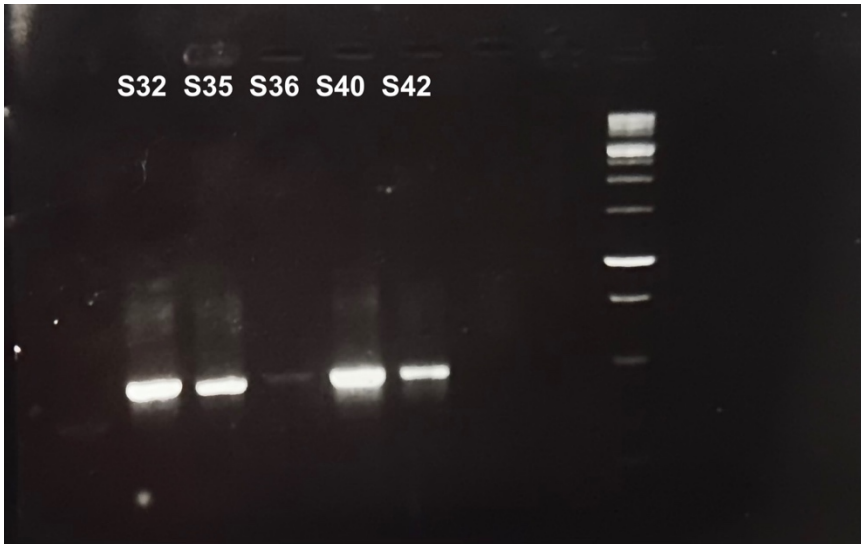

Figure 36: PCR amplification of the *aac(6')-ib* gene

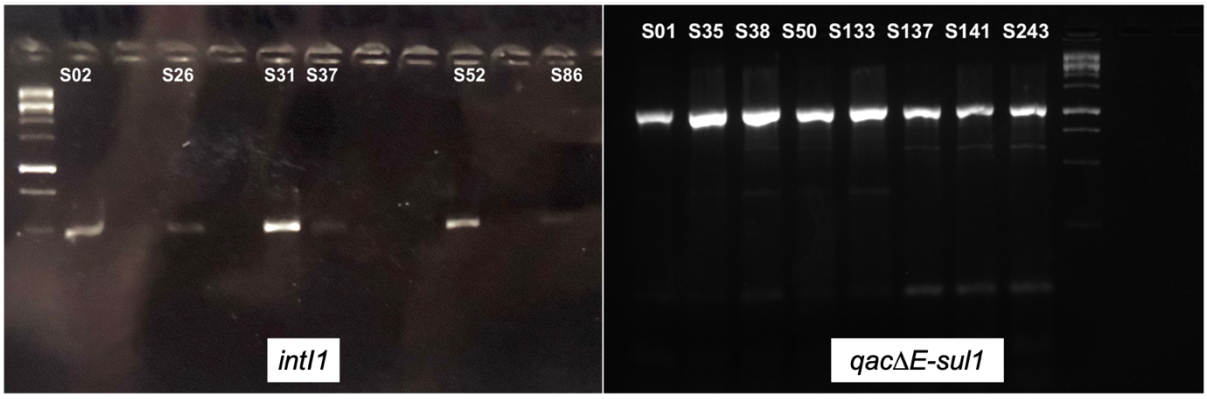

Figure 37: PCR amplification of the *intI1* gene and the *qacΔE-sul1* segment

8.2. Molecular characterization of CL-R isolates

8.2.1. Detection of *mcr* genes:

The PCR results indicated that all the CL-R strains (n=54) carried the *mcr-1* gene (100 %), while the other *mcr* genes were absent (Figure 38).

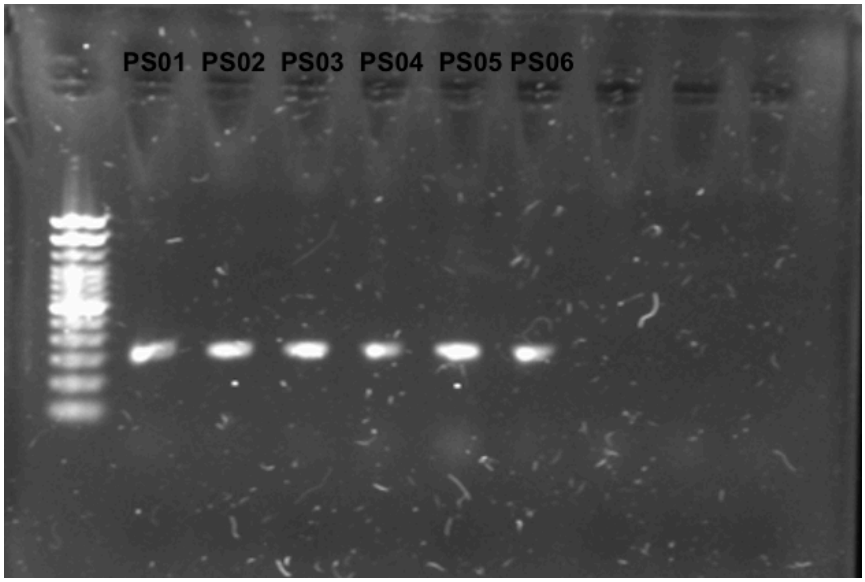

Figure 38: PCR amplification of the *mcr-1* gene

8.2.2. Whole genome sequencing (WGS):

The isolates displayed various resistance determinants and plasmids and belonged to different sequence types (STs). The molecular characteristics of the sequenced strains are represented in Table S5.

a. Resistant determinants:

The isolates carried different antibiotic-resistance genes. The colistin-resistance was only encoded by the *mcr-1* gene in all strains (100 %). Four narrow-spectrum β-lactamase genes were identified, with $bla_{\text{TEM-1B}}$ being the most frequent (56 %), followed by $bla_{\text{TEM-1A}}$ at a 6 % rate and $bla_{\text{TEM-1C}}$ and $bla_{\text{SHV-1}}$, both at a 2 % rate. Four tetracycline resistance genes were detected: *tet(A)*, *tet(B)*, tet(M), and *tet(D)* at 85 %, 8 %, 4 %, and 2 % rates, respectively. Fluoroquinolones resistance was mainly conferred by chromosomal mutations occurring in 11 quinolone resistance determining regions (QRDRs), namely *gyrA* S83L (98 %), *gyrA* D87N (78 %), *gyrA* D87Y (6 %), *parC* S80I (80 %), *parC* E84A (11 %), *parC* E84K (4 %), *parC* E84G (4 %), *parC* S56T (4 %), *parC* S57T (2 %), *parE* S458A (13 %), and *parE* I355T (4 %). The plasmid-mediated fluoroquinolone resistance was also identified in two isolates that carried the *qnrS1* gene (4 %) concurrently with the QRDRs mutations. Six aminoglycoside-resistance genes were detected, *aadA1* (20 %), *aadA2* (19 %), *aadA2b* (6 %), *aph(3')-Ia* (30 %), *aph(3")-Ib* (11 %), and *aph(6)-Id* (13 %). Furthermore, trimethoprim resistance was

associated with four genes: *dfrA1* (7 %), *dfrA7* (2 %), *dfrA12* (9 %), and *dfrA14* (33 %), whereas sulfonamide resistance was attributed to *sul1* (13 %), *sul2* (33 %), and *sul3* (20 %) genes. Two macrolide resistance genes: *mph(A)* (44 %) and *mph(B)* (2 %), and three chloramphenicol resistance genes: *cmlA1* (11 %), *catA1* (15 %), and *floR* (2%), were identified. Finally, only one fosfomycin resistance gene, *fosA4* (2 %), was detected.

On the other hand, some strains also carried disinfectant resistance genes. The *qacL* and *qacE* genes associated with quaternary ammonium compounds (QACs) resistance were detected at 17 % and 19 % rates respectively. In addition, the hydrogen peroxide resistance was mediated by the *sitABCD* determinant which was present in 72 % of the isolates (Figure 39).

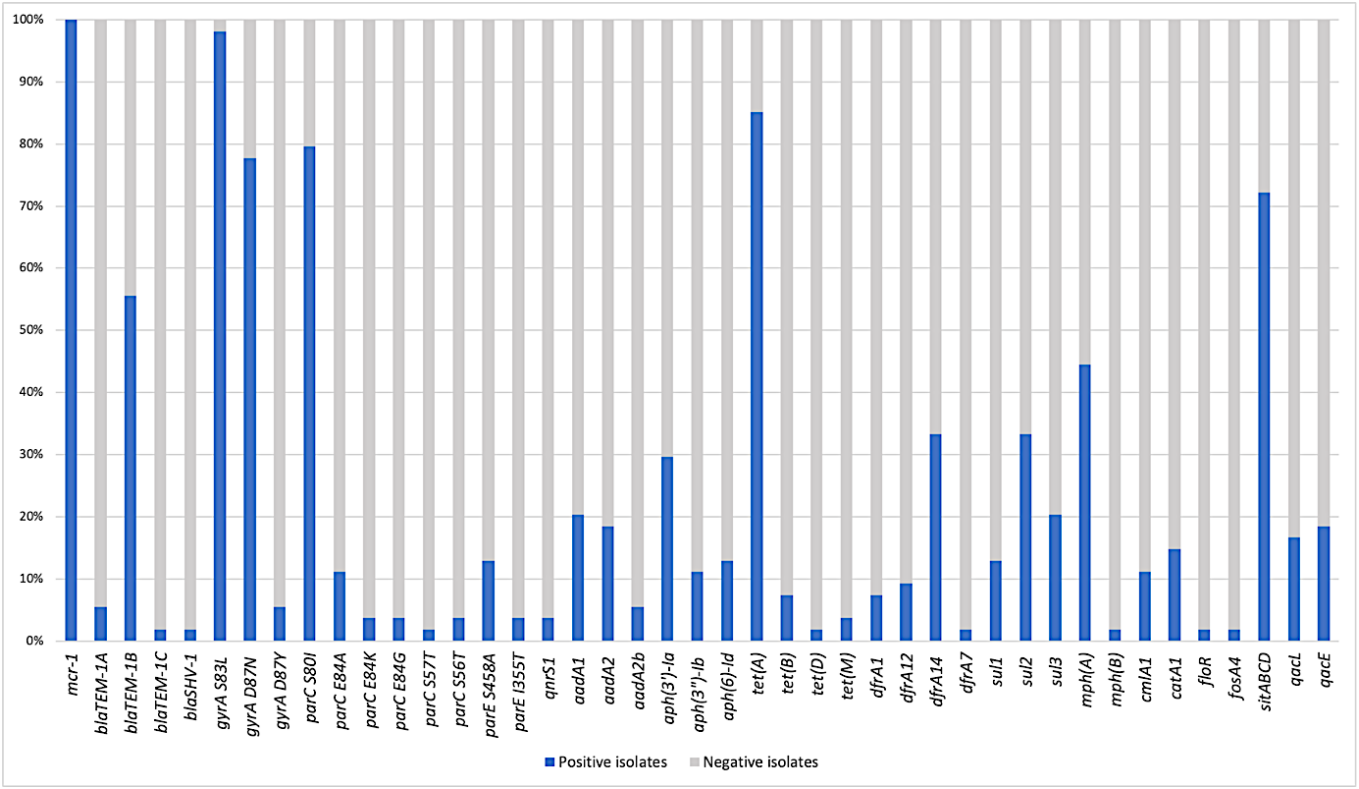

Figure 39: Resistance determinants of the CL-R isolates

b. Plasmids:

Twenty-three plasmid incompatibility groups were identified, each sequenced strain harbored between 2 and 8 plasmids. The *mcr-1* gene was exclusively carried on IncI2 (delta) plasmid in all strains (100%). Besides, other plasmids were present at different frequencies; IncI1-I (19%), IncI1-I (alpha) (24%), IncFIA (11%), IncFIB (81%), IncFIC (54%), IncFII (19%), IncFII (pCoo) (2%), IncFI1 (2%), IncFIB(pLF82) (7%), IncY (6%), IncX1 (15%),

IncX4 (2%), IncHI2 (6%), IncHI2A (4%), IncQ1 (7%), IncR (2%), IncB/O/K/Z (2%), p0111 (31%), Col156 (7%), Col8282 (9%), ColpVC (9%), and ColpEC648 (4%) (Figure 40).

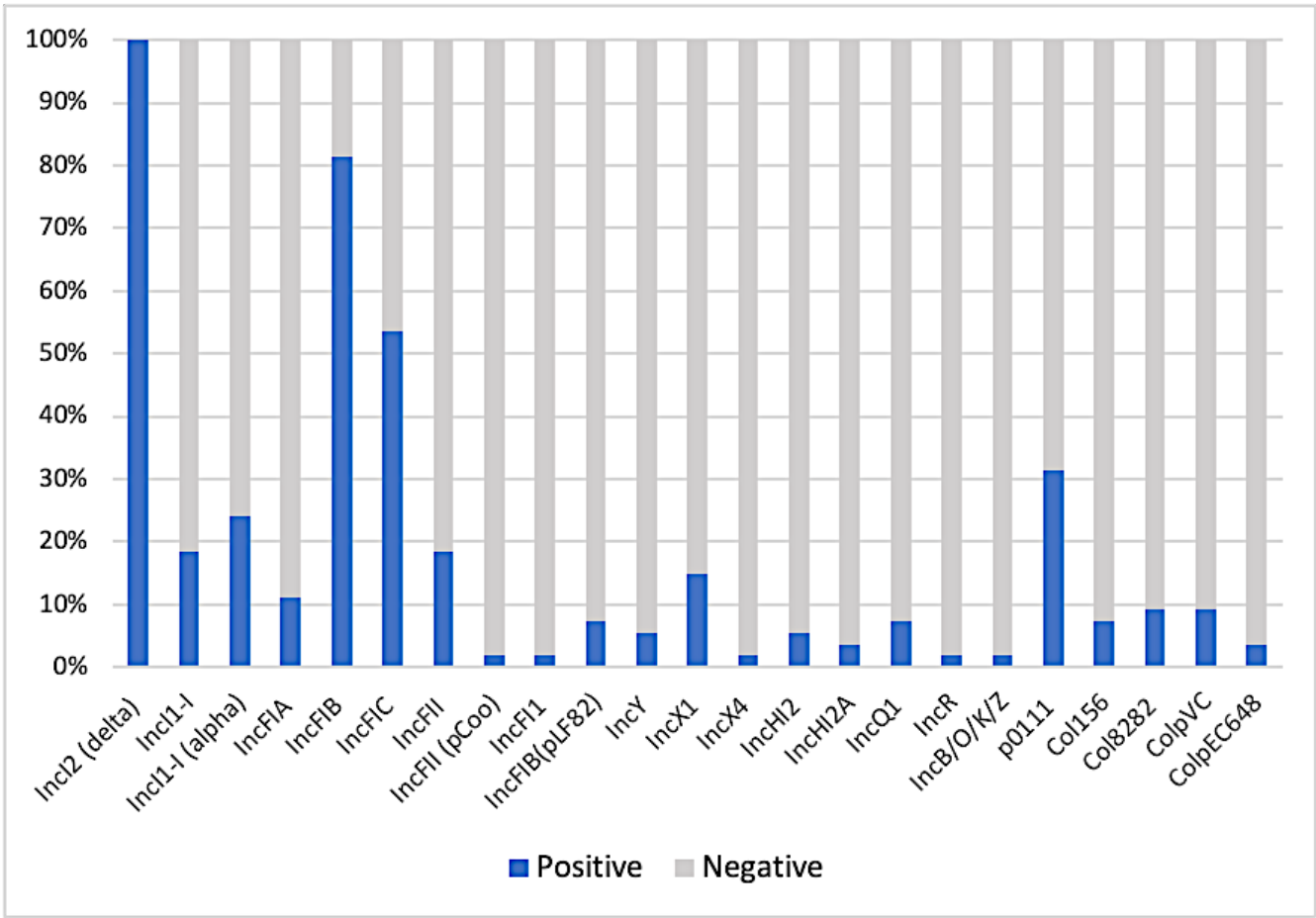

Figure 40: Plasmid incompatibility groups of the CL-R isolates

c. Multi-locus sequence typing (MLST):

The sequenced strains belonged to 28 different ST lineages, with some STs being detected multiple times within the same and/or in different farms (Figure 41).

♦ **Farm F06.p:**

- **1st sampling campaign:** Three different lineages, ST168, ST162 and ST6756, were detected in chicken, rooster and duck feces, respectively.

- **2nd sampling campaign:** Eleven STs were identified in this sampling campaign, ST117, ST93, ST115, ST189 and ST770 in chicken, ST23 and ST354 in drinking water,

ST162 and ST453 in wastewater, ST1485 in rooster feces and ST3941 in duck feces. The ST189 and ST162 were also detected in a wall swab and a soil sample respectively.

- **3rd sampling campaign:** Only two STs were found in this sampling; ST297 in drinking water and ST93 in soil.

♦ **Farm F07.p:**

- **1st sampling campaign:** ST7518 and ST7139 were detected in chicken feces
- **2nd sampling campaign:** Only ST155 was detected in a chicken sample
- **3rd sampling campaign:** ST3941 was found in soil

♦ **Farm F08.p:**

- **1st sampling campaign:** Three STs lineages were identified in chicken, ST744, ST162 and ST1011
- **2nd sampling campaign:** ST354, ST162 and ST224 were detected in chicken samples
- **3rd sampling campaign:** Two different STs were found in soil samples; ST226 and ST11956
- **4th sampling campaign:** Two ST lineages ST616 and ST101 were identified in the fourth campaign, both in chicken, drinking water, and wall swabs, while the ST101 was further detected in a soil sample.

♦ **Farm F01.p:**

Four different STs were detected in this farm in chicken samples, the ST93, ST648, ST1640 and ST58.

♦ **Farm F09.p:**

Only ST8492 was identified in farm F09.p in chicken and soil samples.

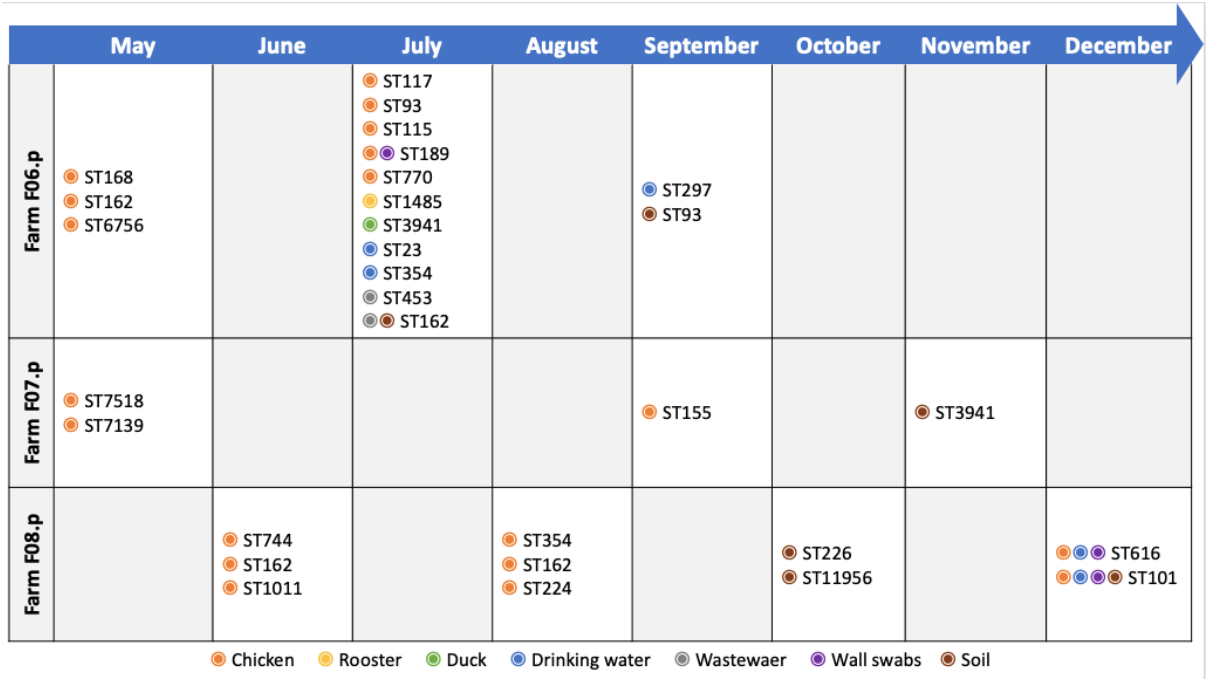

Figure 41: MLST of the strains detected during the longitudinal surveillance of farms F06.p, F07.p, and F08.p

d. Single nucleotide polymorphism (SNP)

Notably, several strains showed low SNP variation (≤20-30) suggesting a genetic relatedness. These strains were mainly recovered from the same farm and the same sampling campaign (Figure 42). Nonetheless, four strains isolated from farm F08.p at different sampling times belonged to ST162 and displayed low SNP differences: PS36 and PS42 from the first and the second sampling campaigns respectively differed by 12 SNPs, while PS38 from the first campaign and PS40 from the second campaign differed by 14 SNPs.

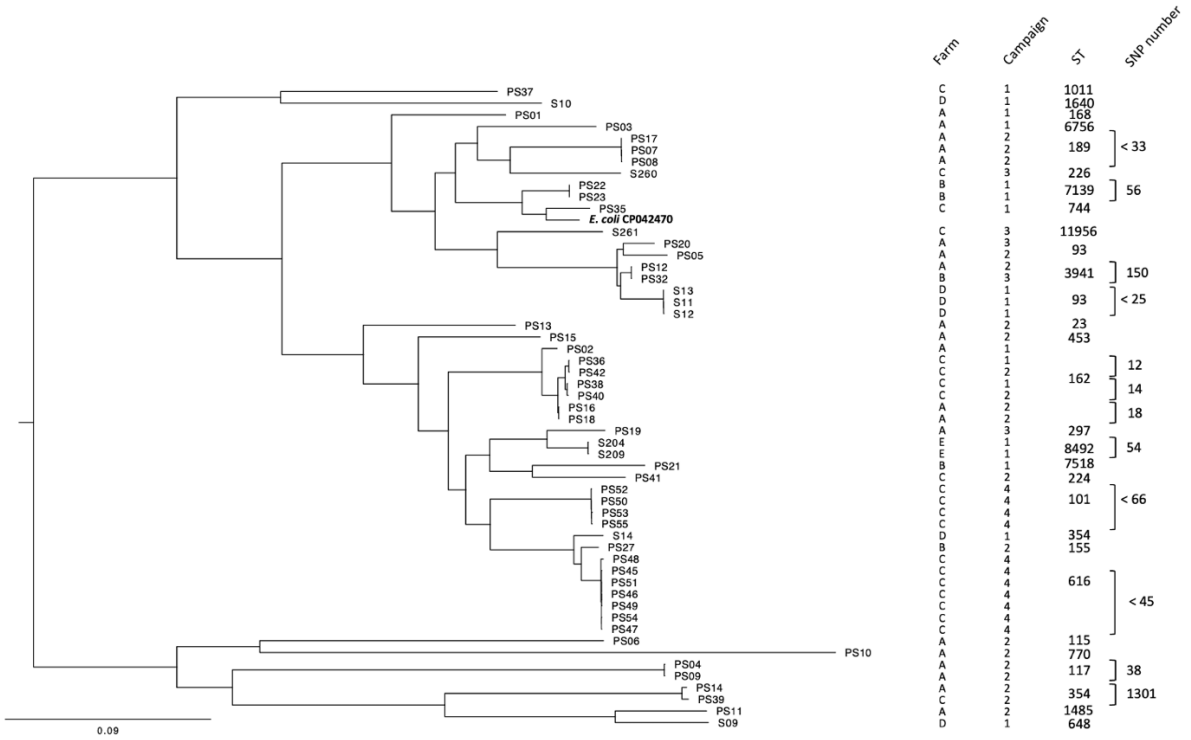

Figure 42: Phylogenetic tree based on single nucleotide polymorphisms (SNPs). *E. coli* strain CP042470 was used as reference.

Discussion

This thesis investigated the dissemination of third-generation cephalosporin-resistant (3GC-R) and colistin-resistant (CL-R) *Escherichia coli* in poultry, bovine and ovine farms in Guelma province, northeastern Algeria. Specifically, we aimed to characterize the phenotypic and molecular resistance profiles of the detected isolates and to evaluate the influence of different factors on their prevalence. Our further objective was to monitor the longitudinal occurrence of colistin-resistant isolates and to assess their potential persistence in poultry farms.

In summary, a total of 112 isolates were detected, including 58 3GC-R and 54 CL-R *E. coli* across 21 of the 38 sampled farms. Strains were isolated from various sample types at different rates, including animal and environmental samples. The prevalence of the detected strains exhibited a notable variation that was mainly influenced by the animal hosts, the geographical region, and the farming system, highlighting the complex, multifactorial nature of the spread of resistant bacteria among farm animals.

Interestingly, various phenotypic resistance patterns were identified in this study, accompanied by diverse genetic profiles and molecular resistance determinants. The isolates displayed high resistance rates against a wide range of antibiotics and carried multiple genes encoding resistance to antibiotics and disinfectants as well as interesting chromosomal mutations.

The 3GC-R *E. coli* isolates were detected in poultry, bovine, and ovine farms, with the highest prevalence observed in chicken, followed by cattle, and the lowest in sheep farms. These findings underscore the ubiquitous spread of cephalosporin resistance among multiple livestock hosts. Although data on the use of cephalosporins in the sampled farms were not available, this widespread distribution of 3GC resistance is likely attributed to the selective pressure applied by the extensive and long-standing use of β-lactams in both veterinary and human sectors (Kim et al., 2023). Nonetheless, other indirect transmission routes might be involved, such as direct human-animal contact, contaminated water and shared environments. Additionally, the high mobility of plasmids carrying β-lactamase genes further facilitates their transferability across multiple bacterial lineages and animal species (Brooks & Jawetz, 2013).

The high prevalence of 3GC resistance in poultry farms is consistent with several studies in Algeria and worldwide, reflecting the wide use of β-lactams in avian production (Chenouf et al., 2021; Moawad et al., 2018; Shirakawa et al., 2020). On the other hand, 3GC resistance

is well established in bovine farms but less frequent in the ovine ones, aligning with previous studies and indicating the higher antibiotic exposure of cattle compared to small ruminants (Tello et al., 2020). However, it should be mentioned that the number of sampled ovine farms in this study is much lower than the number of bovine farms, limiting the comparison's validity.

In contrast, colistin resistance was only observed in poultry farms. This sector-specific dissemination is mainly due to the extensive use of colistin in poultry production for therapeutic and prophylactic purposes, while it remains rare in ruminants (Jansen et al., 2022). These findings concur with previous reports, providing evidence for poultry being the predominant reservoir of colistin resistance (Chabou et al., 2019; Shen et al., 2016).

The prevalence of positive farms fluctuated according to their geographical distribution, where farms located in urban regions displayed a higher prevalence compared to those in the rural zones. Yet, it should be noted that the number of urban farms sampled in this study is much lower than the rural ones. Nonetheless, this disparity was previously reported, and it is likely due to the anthropogenic contamination enabling clonal dissemination of resistant bacteria to the farm environment (Gunasekara et al., 2024; Sanou et al., 2022). This phenomenon is accentuated by the high population density in the urban zones and the intensive human activity, including household and healthcare settings, food vendors and restaurants, water and waste treatment, movement of people and vehicles, and small-scale industries, all of which constitute potential reservoirs and vectors for antibiotic resistance (Gelalcha et al., 2024; Sanou et al., 2022).

A notable variation of the prevalence of positive farms was also observed across different farming systems. Intensive farms showed much higher positivity than the ones managed under a semi-extensive system. This difference is deriven by several factors influencing animals reared in intensive conditions, particularly chickens, such as the high animal density and their proximity, the short production cycles, and often inadequate hygiene and ventilation practices (Gelaude et al., 2014; Tello et al., 2020; Zhao et al., 2021). Altogether, these conditions increase animal's vulnerability to disease outbreaks and compromise the production efficiency, leading to recurrent antibiotic use thereby accelerating the emergence and spread of resistance within the farm environment (Gelaude et al., 2014).

The antibiotic susceptibility profiles detected in this study showed alarming levels of resistance among both 3GC-R and CL-R isolates, despite their different spectra of resistance.

The isolates also displayed high percentages of multidrug resistance, supporting previous reports of the increasing global emergence of MDR bacteria among livestock (Hassen et al., 2019; Zhao et al., 2022).

The 3GC-R isolates were highly resistant to critical β-lactam drugs, including second-, third- and fourth-generation cephalosporins, the monobactam aztreonam, and, at a lower but concerning level, carbapenems despite their exclusion from veterinary practices. These results are concordant with several studies indicating the broad β-lactam resistance among food-producing animals (Tello et al., 2022; Zhao et al., 2022). Remarkably, all carbapenem-resistant strains were isolated from urban intensive farms, reflecting their possible spread from the human environment and/or their co-selection driven by cephalosporin resistance pressure. In addition, 3GC-R isolates exhibited high resistance rates against fluoroquinolones, tetracyclines, and trimethoprim-sulfamethoxazole. This co-resistance was previously reported and is probably due to the wide use of these drugs in livestock production coupled with the common co-location of their resistance determinants on the same plasmids (Mandujano et al., 2023; Tello et al., 2020).

On the other hand, the CL-R isolates showed high resistance levels against fluoroquinolones, tetracyclines, and trimethoprim-sulfamethoxazole but displayed low resistance rates to β-lactams and aminoglycosides. The antimicrobial susceptibility profiles found herein coincide with previous studies (Al Mana et al., 2022; Badr et al., 2022). Colistin, fluoroquinolones, and tetracyclines are commonly used in poultry production for prophylactic treatment and growth promotion. Furthermore, the phenotypic resistance profiles strongly correlated with the data obtained from the selected farms F06.p, F07.p and F08.p, revealing the use of colistin in all three farms in association with doxycycline (in farms F06.p and F07.p) and with ofloxacin (in farm F08.p) for prophylactic and therapeutic purposes, which might explain the high resistance frequencies shown against those antimicrobial agents.

The isolates displayed various resistance determinants, however, their direct comparison is limited since only selected genes were targeted by PCR for the 3GC-R isolates, whereas the whole-genome sequencing (WGS) performed on the CL-R strains provided the complete genetic profiles, including all resistance genes, chromosomal mutations, and plasmids.

Nonetheless, diverse resistance genes were detected in 3GC-R isolates, aligning with their phenotypic resistance profiles. The *bla*$_{\text{CTX-M}}$ gene was unsurprisingly the most frequently

detected; it was identified in poultry, cattle, and sheep samples, which correlates with its global epidemiology and widespread distribution (Hayer et al., 2022; Mandujano et al., 2023). The *bla*$_{\text{CTX-M}}$ gene was broadly reported in Algeria from animal sources, particularly in poultry (Belmahdi et al., 2016; Chenouf et al., 2021). However, there are very few reports on its presence in ruminants (Yousfi et al., 2024), which is most likely due to the lack of studies in this sector compared to the poultry domain.

Interestingly, two carbapenemase genes were detected in our study across four bovine farms; *bla*$_{\text{NDM}}$ in ten isolates and *bla*$_{\text{OXA-181}}$ in one isolate. These genes were previously reported in animals, including cattle, all over the world (Carfora et al., 2022; Tello et al., 2022). In Algeria, carbapenemase genes were identified in companion animals, animal products, and wildlife (Bachiri et al., 2018; Bouaziz et al., 2018; Yaici et al., 2016; Yousfi et al., 2016). A previous study also reported the presence of *bla*$_{\text{OXA-48}}$ -carrying *Klebsiella pneumoniae* in Algerian bovine and ovine farms (Mairi et al., 2019). However, as far as we know, this is the first report of carbapenemase-producing *E. coli* in bovine farms. The emergence of carbapenemase-carrying strains among food-producing animals is alarming, given the great importance of carbapenem antibiotics in human medicine (WHO, 2024b). Furthermore, this finding raises concerns about the circulation of resistant isolates and their genetic determinants between human and animal environments and the great risk of this cross-linked spread to public health.

AmpC genes were also identified in this study, only in cattle, with *bla*$_{\text{CMY}}$ gene being the most prevalent and the *bla*$_{\text{DHA}}$ gene detected in only one isolate. AmpC genes, particularly the *bla*$_{\text{CMY}}$, were increasingly reported in animal samples worldwide, including livestock (Börjesson et al., 2016; Collis et al., 2022; Nossair et al., 2022; Tello et al., 2020). Several studies described AmpC-positive bacteria in animals in Algeria (Belmahdi et al., 2016; Yousfi et al., 2016). Yet, to the best of our knowledge, this is the first detection of AmpC genes in Algerian bovine farms. These findings highlight the wide diversity of β-lactamase genes circulating across animal niches, compromising the efficiency of one of the most frequently used class of drugs in veterinary medicine and posing serious health and economic concerns.

Moreover, additional resistance genes were detected in the 3GC-R isolates, namely the *tetA*, *tetB*, and *aac(6')-Ib* genes, aligning with the phenotypic resistance to tetracyclines and aminoglycosides displayed by the strains and coinciding with previous studies (Jiang et al.,

2025; Yousfi et al., 2024). However, owing to the lack of sequencing, we were not able to investigate the presence of the *aac(6')-Ib-cr* variant, further providing resistance against fluoroquinolones and which is commonly reported in ESBL/AmpC-carrying *E. coli* from different sources (Guillard et al., 2014; Han et al., 2009).

The *int1* gene, encoding for the type 1 integron, was identified in ten isolates, five of which further amplified the conserved region *qacEΔ1-sul1*. The class 1 integron is often located on plasmids and other mobile genetic elements and thus plays a crucial role in the spread of antibiotic resistance genes among bacterial populations in humans and animals (Stalder et al., 2012). The *qacEΔ1-sul1* segment confers both sulfonamide resistance and tolerance to quaternary ammonium compounds, and therefore favors the persistence of bacteria in the farm environment despite disinfection practices (Stalder et al., 2012). Similar findings have been reported in livestock isolates all over the world, confirming the contribution of integrons in the spread and persistence of resistance among food-producing animals (Jiang et al., 2025).

The CL-R strains exhibited different resistance determinants, including antibiotic-resistance and disinfectant-resistance genes, chromosomal mutations, and plasmids. Notably, the *mcr-1* gene was present in all the CL-R isolates. The *mcr-1* gene was first described in animals and its prevalence is known to be higher in livestock which suggests that farm animals, especially chickens, are the main reservoir of this gene (Chabou et al., 2019; Liu et al., 2016; Mmatli et al., 2022; Rhouma et al., 2016). This might be attributed to the extensive use of colistin in this sector, as suggested by (Shen et al., 2016), after the retrospective detection of *mcr-1* in three *E. coli* strains isolated from poultry in the 1980s in China when they first started to use colistin in Chinese farms. In Algeria, the first report of *mcr-1* gene was in an avian *E. coli* strain in 2015 (Olaitan et al., 2016). However, (Berrazeg et al., 2016) have reported an earlier emergence of this gene in a clinical isolate in 2011. It has thereafter been reported in other sources including clinical samples (Yanat et al., 2016), chicken meat (Chaalal et al., 2021), vegetables (Chelaghma et al., 2022), water environment (Berrazeg et al., 2019), farm environment (Touati et al., 2020) and wild animals (Bachiri et al., 2018).

The majority of *mcr-1*-carrying strains also coharbored fluoroquinolone and tetracycline resistance determinants, which aligns with their phenotypic susceptibility profiles. Other antimicrobial resistance genes were found at lower rates, including β-lactams, aminoglycosides, sulfonamides, macrolides, fosfomycin, and chloramphenicol resistance determinants. Several studies have previously reported the coexistence of the above genes in

mcr-1-carrying strains from poultry sources (Al-Mustapha et al., 2022; Moawad et al., 2018; Perreten et al., 2016). Such a phenomenon may increase the dissemination of these strains and enable their persistence (El-Sayed Ahmed et al., 2020).

Furthermore, genes associated with disinfectant resistance were also identified, namely *qacL* and *qacE*, responsible for resistance to quaternary ammonium compounds, and *sitABCD*, which confers hydrogen peroxide resistance. These genes were previously detected in *E. coli* strains isolated from chicken feces and chicken meat (Al-Mustapha et al., 2022; Kassem et al., 2023). It is noteworthy that hydrogen peroxide and quaternary ammonium compounds are widely used in poultry farms to sanitize chicken houses and equipment, as was the case for farm F06.p. The carriage of disinfectant-resistance genes in MDR and *mcr-1*-positive bacteria is of particular concern as it complicates their elimination during the disinfection process and enhances the risk of their long-term persistence in the farm environment.

Various plasmids were detected in colistin-resistant strains. Although the *mcr-1* gene can be carried on many incompatibility groups of plasmids, it was exclusively spotted on IncI2 (delta)-type plasmid in all strains despite their origin. This plasmid type was previously detected in *mcr-1* positive *E. coli* strains isolated from chicken feces in Lebanon (Abou Fayad et al., 2022). Ding et al., 2021 has also reported this plasmid type in *mcr-1* positive *E. coli* in human feces as well as in retail raw milk during the same period in Singapore suggesting the possible dissemination of *mcr-1* via this plasmid. On the other hand, Strepis et al., 2021 has described an interspecies transmission of IncI2 (delta) plasmid among three clinical species isolated from the same patient, namely *E. coli*, *K. pneumoniae* and *K. georgiana*. Moreover, the IncI2 (delta) plasmid was recently identified in *mcr-1* carrying *E. coli* from camels in United Arab Emirates (Ghazawi et al., 2024) as well as in non-*mcr-1 -positive E. coli* in chicken meat (Kurittu et al., 2021) and in cattle composite feces (Kim et al., 2022). However, to the best of our knowledge, this is the first detection of IncI2 (delta) plasmid in Algeria.

Additionally, IncF plasmids, mainly IncFIB and IncFIC, were the second most predominant plasmid type in this study, which is not surprising since this type is the most commonly detected in *E. coli* from animal and human sources across the world (Rozwandowicz et al., 2018). Other plasmids were identified in the strains as well, namely IncI1, IncX, IncY, IncH, IncQ, IncB/O/K/Z, p0111 and Col plasmids. Those plasmids were also described in poultry and other livestock in previous studies (Al-Mustapha et al., 2022; Kassem et al., 2023; Khine et al., 2022; Li et al., 2020; Shirakawa et al., 2020).

Overall, twenty-eight different *E. coli* STs were detected in this study, with several observed multiple times over the longitudinal sampling period. This high diversity of genotypes indicates the polyclonal dissemination of different *mcr-1-harboring* strains among poultry farms.

E. coli ST162 and ST93 were the most frequent genotypes in our study, and they were isolated at different sampling times in farms F06.p and F08.p. However, this finding is not sufficient to determine whether these strains persisted in the farm environment over the production cycles or were reintroduced with the new flocks. Furthermore, some STs were spotted in different samples at the same time in one farm, as was the case of ST101 and ST616 being isolated in farm F08.p from chicken, drinking water, wall swabs, and soil simultaneously, suggesting a clonal dissemination of these strains among different niches within the same farm.

Of particular interest, four strains belonging to ST162 were detected across different sampling campaigns on the same farm and displayed a pairwise SNP of 12 and 14. This low divergence indicates a clonal relatedness between the isolates. It suggests a within-farm persistence of *mcr-1*-carrying *E. coli* lineages, potentially reintroduced into new flocks from a common reservoir (contaminated water, soil, etc.) despite the flock's rotation. This finding highlights the need for adequate decontamination of the farm environment between production cycles.

Two isolates of ST3941 with an SNP of 150 were present in two different farms, F06.p and F07.p, during two different periods. This high SNP count indicates that the strains are not genetically related and thus excludes the hypothesis of clonal transmission between the farms. However, the two farms are relatively close (600 meters apart) and surveyed by the same veterinarian, which might suggest a common contamination source. These results indicate that several *mcr-1-carrying E. coli* clones are disseminating in this area, evolving, and showing a strong phylogenetic structure.

Several studies have previously reported the circulation, in Algeria, of *mcr-1*-carrying *E. coli* belonging to some of the ST lineages identified herein. The ST101 and ST224 genotypes were the most described; they were detected in vegetables and chicken meat, respectively, as well as in white stork and wastewater (Chaalal et al., 2021; Chelaghma et al., 2022, 2022; Cherak et al., 2022). Besides, *E. coli* ST93 and ST155 were also reported in wastewater and hospital tap water respectively (Cherak et al., 2022).

The overall findings of this thesis carry important implications for public health, veterinary medicine, and environmental safety. The coexistence of extended-spectrum β-lactamase (ESBL), AmpC, carbapenemase, and *mcr-1* genes in livestock-associated *E. coli* illustrates the scale of the antibiotic resistance threat in Algeria. The detection of novel *mcr-1*-carrying sequence types (STs), plasmid-mediated carbapenemases in cattle, and disinfectant resistance genes in poultry-associated isolates highlights that livestock farms are not only reservoirs but also amplifiers of critical resistance determinants.

The results of this thesis strongly support a One Health framework for antibiotic resistance surveillance and control. The overlap between sequence types isolated from livestock and farm environments in this study and those from vegetables (Chelaghma et al., 2022), wastewater (Loucif et al., 2022), and wildlife (Bachiri et al., 2018) in Algeria illustrates how resistant strains move freely across human, animal, and environmental compartments. Poultry farms, in particular, appear to act as hotspots for the amplification and dissemination of *mcr-1*-positive *E. coli*, which may then spread to other reservoirs.

While this thesis provides valuable insights into the epidemiology of 3GC-R and CL-R *E. coli* in Algerian livestock, several limitations must be acknowledged. These limitations should be taken into account for an accurate interpretation of the findings and for guiding future research.

First, at the sampling level, the restricted study area and limited access to farms constrained the ability to truly estimate the prevalence of 3GC-R and CL-R isolates within the studied region. Although samples were collected from 38 farms, the number of isolates per species and per farm varied considerably, which may have biased prevalence estimates and restricted meaningful statistical comparisons across species and the in-depth evaluation of prevalence variation according to different factors such as animal species, farming system and geographical location. A larger and more balanced sampling design is therefore needed to accurately capture the resistance dynamics across different livestock species and assess the influence of farming and geographical factors on the prevalence fluctuation.

Second, the geographical coverage of the study was limited, since sampling was restricted to farms in Guelma, which may not reflect the diversity of farming practices, climates, and antibiotic usage in other regions of the country. Expanding surveillance to additional provinces would be necessary to generate a more representative national overview

of livestock-associated antibiotic resistance in Algeria. Similarly, the longitudinal investigation of colistin-resistance in poultry farms was restricted by the difficulty in obtaining farmers cooperation, hindering the ability to conduct a more extended surveillance and limiting the understanding of the long-term dynamics of resistant isolates within the farm environment. Extended longitudinal monitoring across multiple production cycles and seasons would help clarify the temporal dynamics of resistance.

Third, data on antibiotic usage and disinfection protocols were incomplete. Although some information was obtained for poultry farms, systematic and quantitative records were not available. This gap hindered the ability to directly link antibiotic usage and disinfection practices with resistance outcomes. Future studies should aim to integrate detailed farm-level data, ideally in collaboration with veterinary authorities and farmers, and within the framework of national surveillance.

Furthermore, although environmental samples were included, the broader ecological context was not deeply explored. Important contributors to resistance circulation, such as effluents, wild birds, urban wastewater and individuals involved in the farm activities should be included. Addressing these factors in future studies would provide a more complete picture of the cross-sectoral dissemination of resistant isolates and their genes.

At the practical level, the pooling approach was restrictive yet unavoidable, given the large number of samples collected. While this step was essential to ensure practical feasibility and reduce laboratory workload, it inevitably limited the analytical resolution by potentially concealing individual variations among samples.

Finally, in the detection of resistance determinants, only a limited set of genes was targeted by PCR for 3GC-R isolates, which may have underestimated the diversity of underlying resistance mechanisms. In contrast, CL-R isolates underwent WGS, offering a more comprehensive genetic profile. This discrepancy limits direct comparisons between the two groups. Future investigations should apply WGS systematically to all isolates in order to capture the full spectrum of resistance determinants and plasmid structures.

Despite these limitations, this thesis offers crucial insights into the dynamics and molecular characteristics of 3GC-R and colistin-resistant *E. coli* strains circulating among Algerian livestock. Future investigations should aim to expand the sampling coverage across

different regions and animal species, extend longitudinal surveillance over multiple production cycles, and integrate whole-genome sequencing approaches. Strengthening collaboration between researchers, veterinarians, and farmers will also be essential to generate more comprehensive and relevant data, thereby deepening the understanding of antibiotic resistance circulation and persistence within livestock ecosystems.

Conclusion

Antibiotic resistance represents one of the most serious challenges to public health, threatening both human and animal populations and compromising the effectiveness of critical therapeutic agents. While livestock production plays a central role in global food security and economic sustainability, it also serves as a significant reservoir for the amplification and spread of antibiotic-resistant bacteria and their genetic determinants.

To address this concern, the present thesis conducted a comprehensive investigation of the dissemination of third-generation cephalosporin-resistant (3GC-R) and colistin-resistant (CL-R) *Escherichia coli* among poultry, bovine and ovine farms. By combining phenotypic and molecular analyses with longitudinal sampling in poultry farms, this study has provided crucial insights into the prevalence, resistance mechanisms as well as patterns and genetic diversity of strains circulating in livestock and their environment.

A total of 112 resistant *E. coli* isolates were identified in this study in 21 of the 38 sampled farms, confirming their wide dissemination across the region. Notably, 3GC resistance was found in all animal species investigated, including chicken, cattle and sheep, while colistin resistance was exclusively detected in poultry farms, indicating the sector-specific patterns of antibiotic resistance and highlighting the role of poultry production as a major reservoir of antibiotic resistance. The prevalence of resistant isolates varied across the sampled farms and was mainly influenced by (i) the animal species with a higher rate in poultry farms followed by cattle and then sheep, (ii) the farming system, where intensive farms displayed a higher resistance prevalence compared to the semi-extensive systems and lastly, (iii) the geographical distribution with a higher resistance rate in urban farms compared to the rural regions.

The phenotypic and molecular analyses revealed high rates of multidrug resistance (MDR) and a significant diversity in genetic resistance determinants, including critical antibiotic resistance genes such as *bla*$_{\text{CTX-M}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{OXA-181}}$, *bla*$_{\text{CMY}}$, *bla*$_{\text{DHA}}$ and *mcr-1*, disinfectant resistance genes, epidemic plasmids and the integron type 1. On the other hand, the longitudinal investigation of colistin resistance in poultry farms indicated the polyclonal dissemination of diverse sequence types (STs), including globally prevalent lineages, such as ST162, ST101, ST224 and ST93, which were previously identified in Algeria among clinical, animal and environmental samples. Furthermore, some of these STs were detected multiple times during the sampling period, underscoring their potential persistence in the farm environment despite the disinfection routine between the producing cycles or their reintroduction from a continuous source of contamination.

The relevance of these findings extends beyond the veterinary sector and underlines the interconnection between animal, human and environmental health. Notably, the detection of carbapenemase genes in urban farms despite the exclusive use of carbapenems in humans, along with the occurrence of strains with similar phenotypic and genotypic resistance profiles in both animals and their environment highlight the widespread propagation of antibiotic resistance across different niches and reinforce the One Health perspective, confirming that this challenge must be addressed through coordinated efforts between human, animal and environmental domains.

These efforts should encompass the implementation of effective stewardship measures in order to monitor the use of antibiotics in both human and veterinary medicine and to tackle their non-therapeutic application in animals. The establishment of harmonized surveillance programs is also needed to ensure a continuous control of the antibiotic use and resistance across countries and provide reliable and comparable data. In terms of preventive measures, the improvement of biosecurity practices and farm hygiene together with appropriate vaccination are effective actions to reduce animal vulnerability to infections and thus limit antibiotic demand.

From a methodological perspective, this thesis has revealed several gaps that should be addressed in future research. Improving of the sampling strategy by expanding the study area to other Algerian regions, broadening the sample range to other animal species, human and food samples, integrating a systematic data collection regarding antibiotic use and disinfection and extending the longitudinal surveillance period would provide a more comprehensive and general picture of the antibiotic resistance dynamics at the national level. Furthermore, performing whole-genome sequencing (WGS) on all isolates would allow for a better understanding of resistance mechanisms, plasmid diversity and genetic relatedness between strains and therefore help assessing the routes of transmission and the potential persistence of resistant isolates. Overcoming these limitations will enhance the insights into the antibiotic resistance patterns at both national and global levels.

In conclusion, the present study demonstrates that livestock production in Algeria serves as a significant reservoir for resistant bacteria harboring critical genes and mobile genetic elements. These findings emphasize the extent of antibiotic resistance challenge within animal, human and environmental sectors and thus underscore the urgent need for effective surveillance and mitigation measures aligning with the global One Health strategy. The

implementation of such integrated and cross-sectoral efforts is essential to improve the resistance monitoring across all domains, promote global health security and ensure the livestock production sustainability in Algeria and worldwide.

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Supplementary material

Table S1: Poultry farms and samples

Farm	Location	Type of the region	Farming system	Date of sampling	Age of animals	Number of animals	Type of samples	Number of samples
F01.p	**Heliopolis**	Rural	Intensive	**February 2022**	39 days	5000	Chicken feces	30
F02.p	**Medjez Ammar**	Rural	Intensive	**February 2022**	27 days	2500	Chicken feces	30
F03.p	**Heliopolis**	Rural	Intensive	**March 2022**	30 days	3800	Chicken feces	30
F04.p	**Guelaât Bou Sbaâ**	Rural	Intensive	**April 2022**	46 days	2500	Chicken feces	30
F05.p	**Guelaât Bou Sbaâ**	Rural	Intensive	**April 2022**	21 days	3000	Chicken feces	30
F06.p	**Bendjerrah**	Rural	Intensive	**May 2022**	51 days	3800	Chicken feces	40
							Rooster feces	02
							Duck feces	02
				July 2022	30 days	4000	Chicken feces	40
							Rooster feces	02
							Duck feces	02
							Drinking water	02
							Wastewater	02
							Wall swabs	02
							Chicken food	02
							Soil	02
				September 2022	38 days	3400	Chicken feces	40
							Rooster feces	02
							Duck feces	02
							Drinking water	02
							Wastewater	02
							Wall swabs	02
							Chicken food	02
							Soil	02
F07.p	**Bendjerrah**	Rural	Intensive	**May 2022**	50 days	1900	Chicken feces	30
				September 2022	25 days	3800	Chicken feces	30
							Drinking water	02
							Wastewater	02
							Wall swabs	02
							Chicken food	02
							Soil	02
				November 2022	21 days	4000	Chicken feces	30
							Drinking water	02
							Wastewater	02
							Wall swabs	02
							Chicken food	02
							Soil	02
F08.p	**Medjez Ammar**	Rural	Intensive	**June 2022**	28 days	4000	Chicken feces	30

				August 2022	28 days	4000	Chicken feces	30
							Drinking water	02
							Wastewater	02
							Wall swabs	02
							Chicken food	02
							Soil	02
				October 2022	No Animals were present		Soil samples	02
							Wall swabs	02
							Food trough swabs	02
							Water trough swabs	02
				December 2022	46 days	4000	Chicken feces	30
							Drinking water	02
							Wastewater	02
							Wall swabs	02
							Chicken food	02
							Soil	02
F09.p	**Medjez Ammar**	Rural	Intensive	**August 2022**	39 days	4000	Chicken feces	30
							Drinking water	02
							Soil	02
F10.p	**Bouati Mahmoud**	Rural	Intensive	**September 2022**	44 days	3500	Chicken feces	30

Table S2: Bovine farms and samples

Farm	Location	Type of the region	Farming system	Date of sampling	Age of animals	Number of animals	Number of samples
F11.b	**Heliopolis**	Urban	Intensive	September 2021	02-05 months	15	08
F12.b	**Heliopolis**	Urban	Intensive	September 2021	01-04 years	09	09
F13.b	**Heliopolis**	Urban	Intensive	September 2021	03-05 years	11	11
F14.b	**Heliopolis**	Urban	Intensive	December 2021	01-07 years	20	18
F15.b	**Heliopolis**	Urban	Intensive	December 2021	04-10 years	06	06
F16.b	**Heliopolis**	Urban	Intensive	December 2021	01-04 years	05	05
F17.b	**Heliopolis**	Urban	Semi-extensive	December 2021	01-05 years	10	10

F18.b	**Bouati Mahmoud**	Rural	Semi-extensive	February 2022	03-05 years	05	05
F19.b	**Bouati Mahmoud**	Rural	Semi-extensive	February 2022	01-04 years	06	03
F20.b	**Bouati Mahmoud**	Rural	Semi-extensive	February 2022	04-06 years	04	04
F21.b	**Bouati Mahmoud**	Rural	Semi-extensive	February 2022	03-07 years	04	04
F22.b	**Bouati Mahmoud**	Rural	Semi-extensive	February 2022	02-08 years	05	05
F23.b	**Hammam Debagh**	Rural	Semi-extensive	March 2022	02-06 years	07	03
F24.b	**Hammam Debagh**	Rural	Semi-extensive	March 2022	01-04 years	20	10
F25.b	**Hammam Debagh**	Rural	Semi-extensive	March 2022	03-06 years	08	04
F26.b	**Bouati Mahmoud**	Rural	Semi-extensive	May 2022	03-05 years	13	10
F27.b	**Bouati Mahmoud**	Rural	Semi-extensive	May 2022	02-03 years	07	05
F28.b	**Bouati Mahmoud**	Rural	Semi-extensive	May 2022	01-04 years	06	05
F29.b	**Bouati Mahmoud**	Rural	Semi-extensive	May 2022	06-10 years	08	06
F30.b	**Bouati Mahmoud**	Rural	Semi-extensive	May 2022	05-07 years	06	04
F31.b	**Bouati Mahmoud**	Rural	Semi-extensive	May 2022	06-08 years	12	10

Table S3: Ovine farms and samples

Farm	**Location**	**Type of the region**	**Farming system**	**Date of sampling**	**Age of animals**	**Number of animals**	**Number of samples**
F32.o	**Bouati Mahmoud**	Rural	Semi-extensive	February 2022	05 months – 03 years	51	30
F33.o	**Bouati Mahmoud**	Rural	Semi-extensive	February 2022	08 months – 02 years	44	30
F34.o	**Bouati Mahmoud**	Rural	Semi-extensive	February 2022	04 months – 03 years	88	10

F35.o	**Bouati Mahmoud**	Rural	Semi-extensive	February 2022	06 months – 05 years	35	20
F36.o	**Bouati Mahmoud**	Rural	Semi-extensive	February 2022	09 months – 04 years	20	10
F37.o	**Bouati Mahmoud**	Rural	Semi-extensive	February 2022	03 months – 02 years	33	10
F38.o	**Medjez Ammar**	Rural	Semi-extensive	March 2022	06 months – 04 years	60	30

Table S4 : Phenotypic and molecular profiles of the 3GC-R isolates

Code	Farm	Origin	ESBL test	EDTA test	Resistance profile	Detected genes
S01	F02.b	Cattle	Positive	/	CTX; AMC; AMX; CAZ; FEP; ATM; CIP; OFX; TE; DO; AK; SXT	*bla*$_{CTX-M}$; *tet*A; *intI1*; *qacΔE-sul1*
S02	F35.o	Sheep	Positive	/	CTX; AMC; AMX; FEP; ATM; TE; SXT	*bla*$_{CTX-M}$; *intI1*
S04	F37.o	Sheep	Positive	/	CTX; AMC; AMX; CAZ; FEP; ATM	*bla*$_{CTX-M}$
S20	F02.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{CTX-M}$; *intI1*
S26	F02.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{CTX-M}$; *tet*A; *intI1*
S31	F02.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{CTX-M}$; *tet*A; *intI1*
S32	F17.b	Cattle	Positive	Negative	CTX; AMC; AMX; CAZ; FEP; ATM; ETP; CIP; OFX; TE; DO; CN; AK; SXT	*bla*$_{CTX-M}$; *tet*A; *aac(6')-Ib*
S33	F17.b	Cattle	Negative	Positive	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; ETP; IMP; CIP; OFX; TE; DO; AK; SXT	*bla*$_{CTX-M}$; *bla*$_{NDM-1}$; *bla*$_{CMY}$

S34	F17.b	Cattle	Negative	Positive	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; ETP; IMP; CIP; OFX; TE; DO; AK; SXT	*bla*$_{CTX-M}$; *bla*$_{NDM-1}$; *bla*$_{CMY}$
S35	F17.b	Cattle	Positive	/	CTX; AMC; AMX; CAZ; FEP; ATM; CIP; OFX; TE; DO; CN; AK; SXT	*bla*$_{CTX-M}$; *tet*A; *aac(6')-Ib*; *intI1*; *qacΔE-sul1*
S36	F17.b	Cattle	Negative	/	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; CIP; OFX; SXT	*bla*$_{CTX-M}$; *bla*$_{CMY}$; *aac(6')-Ib*; *intI1*
S37	F17.b	Cattle	Positive	/	CTX; AMC; AMX; CAZ; FEP; ATM; SXT	*bla*$_{CTX-M}$; *intI1*
S38	F17.b	Cattle	Negative	Positive	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; ETP; IMP; CIP; OFX; TE; DO; SXT	*bla*$_{CTX-M}$; *bla*$_{NDM-1}$; *bla*$_{CMY}$; *tet*A; *intI1*; *qacΔE-sul1*
S40	F17.b	Cattle	Negative	/	CTX; AMC; AMX; CAZ; FEP; ATM; CIP; OFX; TE; DO; CN; AK; SXT	*bla*$_{CTX-M}$; *tet*A; *aac(6')-Ib*
S41	F17.b	Cattle	Negative	Negative	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; ETP; CIP; OFX; TE; CN; AK; SXT	*bla*$_{CTX-M}$; *bla*$_{CMY}$; *aac(6')-Ib*

S42	F16.b	Cattle	Negative	Positive	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; ETP; IMP; CIP; OFX; TE; DO; SXT	*bla*$_{\text{NDM-1}}$; *bla*$_{\text{CMY}}$
S43	F16.b	Cattle	Negative	Positive	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; ETP; IMP; CIP; OFX; TE; DO; SXT	*bla*$_{\text{NDM-1}}$; *bla*$_{\text{CMY}}$
S44	F16.b	Cattle	Negative	Positive	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; ETP; IMP; CIP; OFX; TE; DO; SXT	*bla*$_{\text{NDM-1}}$; *bla*$_{\text{CMY}}$
S45	F16.b	Cattle	Negative	Positive	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; ETP; IMP; CIP; OFX; TE; DO; SXT	*bla*$_{\text{NDM-1}}$; *bla*$_{\text{CMY}}$; *tet*B
S46	F16.b	Cattle	Negative	Positive	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; ETP; IMP; CIP; OFX; TE; DO; SXT	*bla*$_{\text{NDM-1}}$; *bla*$_{\text{CMY}}$; *tet*B
S48	F14.b	Cattle	Negative	Positive	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; ETP; IMP; CIP; OFX; TE; DO; SXT	*bla*$_{\text{NDM-1}}$; *bla*$_{\text{CMY}}$
S49	F14.b	Cattle	Negative	Positive	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; ETP; IMP; CIP; OFX; TE; DO; SXT	*bla*$_{\text{NDM-1}}$; *bla*$_{\text{CMY}}$

S50	F14.b	Cattle	Positive	Negative	CTX; AMC; AMX; CAZ; FEP; ATM; ETP; CN; SXT	*bla*$_{CTX-M}$; *intI1*; *qacΔE-sul1*
S52	F11.b	Cattle	Positive	/	CTX; AMC; AMX; FEP; ATM; SXT	*bla*$_{CTX-M}$; *intI1*
S54	F12.b	Cattle	Positive	/	CTX; AMC; AMX; CAZ; FEP; ATM; OFX; TE; DO	*bla*$_{CTX-M}$
S55	F12.b	Cattle	Positive	Negative	CTX; AMC; AMX; CAZ; FEP; ATM; ETP; IMP; CIP; OFX; TE; DO; AK	*bla*$_{CTX-M}$; *bla*$_{OXA-181}$
S86	F25.b	Cattle	Positive	/	CTX; AMC; AMX; FEP; ATM; TE; CN; AK; SXT	*bla*$_{CTX-M}$; *intI1*
S114	F04.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO	*bla*$_{CTX-M}$
S115	F04.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO	*bla*$_{CTX-M}$
S116	F05.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{CTX-M}$
S117	F05.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{CTX-M}$
S118	F05.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; AK; SXT	*bla*$_{CTX-M}$

S119	F05.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; AK; SXT	*bla*$_{\text{CTX-M}}$
S120	F05.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{\text{CTX-M}}$; *intI1*
S121	F05.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{\text{CTX-M}}$; *intI1*
S124	F04.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO	*bla*$_{\text{CTX-M}}$
S126	F04.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO	*bla*$_{\text{CTX-M}}$
S127	F04.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO	*bla*$_{\text{CTX-M}}$
S128	F06.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; AK	*bla*$_{\text{CTX-M}}$; *tet*A
S133	F07.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{\text{CTX-M}}$; *tet*A; *intI1*; *qacΔE-sul1*
S134	F07.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{\text{CTX-M}}$
S135	F07.p	Chicken	Positive	/	CTX; AMC; AMX; CAZ; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{\text{CTX-M}}$; *tet*A; *intI1*

S136	F07.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{CTX-M}$; *tet*B
S137	F07.p	Chicken	Positive	/	CTX; AMC; AMX; CAZ; FEP; ATM; CIP; OFX; TE; DO; CN; AK; SXT	*bla*$_{CTX-M}$; *intI1*; *qacΔE-sul1*
S141	F26.b	Cattle	Positive	/	CTX; AMC; AMX; CAZ; FEP; ATM; TE; DO; SXT	*bla*$_{CTX-M}$; *intI1*; *qacΔE-sul1*
S145	F27.b	Cattle	Positive	/	CTX; AMC; AMX; FEP; ATM	*bla*$_{CTX-M}$
S148	F30.b	Cattle	Positive	/	CTX; AMC; AMX; CAZ; FOX; FEP; ATM; OFX; TE; DO	*bla*$_{CTX-M}$; *bla*$_{DHA}$
S153	F08.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{CTX-M}$; *intI1*
S154	F08.p	Chicken	Positive	/	CTX; AMC; AMX; CAZ; FEP; ATM; CIP; OFX; TE; DO; AK; SXT	*bla*$_{CTX-M}$
S199	F09.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{CTX-M}$
S239	F10.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; CN; AK; SXT	*bla*$_{CTX-M}$
S241	F10.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{CTX-M}$

S242	F10.p	Chicken	Negative	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{\text{CTX-M}}$
S243	F10.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{\text{CTX-M}}$; *intI1*; *qacΔE-sul1*
S244	F10.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; CN; AK; SXT	*bla*$_{\text{CTX-M}}$; *intI1*
S245	F10.p	Chicken	Positive	/	CTX; AMC; AMX; CAZ; FEP; ATM; CIP; OFX; TE; DO; CN; SXT	*bla*$_{\text{CTX-M}}$; *intI1*
S246	F10.p	Chicken	Positive	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{\text{CTX-M}}$; *tet*C; *intI1*
S247	F10.p	Chicken	Negative	/	CTX; AMC; AMX; FEP; ATM; CIP; OFX; TE; DO; SXT	*bla*$_{\text{CTX-M}}$; *tet*C; *intI1*

Table S5 : Phenotypic and molecular profiles of the CL-R isolates

Farms	**Sampling time**	**Code**	**Origin**	**Resistance profile**	**STs**	**Acquired antimicrobial resistance genes**	**Chromosomal mutations**	**Disinfectant resistance genes**	**Plasmids**
	1st	PS01	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	168	*mcr-1, bla*$_{\text{TEM1-B}}$*, aadA1, aadA2, aph(3')-Ia, tet(A), dfrA1, sul1, dfrA12, sul3, mph(A)*	*parC* S80I, *parE* S458A, *gyrA* D87N, *gyrA* S83L	*sitABCD, qacL, qacE*	Col156, IncFIB, IncFII, Incl2(Delta), IncY

		PS02	**Rooster**	AMX, AMC, NA, TE, DO, CL	162	*mcr-1, bla*$_{TEM1-C}$*, tet(A), tet(M), mph(A), fosA4*	*gyrA* S83L	*sitABCD*	IncFIB, IncFIC, IncFII(pCoo), Incl2(Delta), IncY
		PS03	**Duck**	NA, OFX, CIP, TE, DO, CL	6756	*mcr-1, tet(A), dfrA14, qnrS1*	*parC A56T, parC S80I, parE S458A, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFII, IncI2(Delta)
	2nd	PS04	**Chicken**	NA, OFX, CIP, TE, DO, SXT, CL	117	*mcr-1, aph(6)-Id, aph(3')-Ia, tet(B), sul2*	*parC E84K, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFIC, Incl2(Delta)
		PS05	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, CL	93	*mcr-1, bla*$_{TEM1-B}$*, aadA1, aadA2, aph(3')-Ia, tet(A), dfrA1*	*parC* S80I, *parC* S57T, *gyrA* D87N, *gyrA* S83L	*qacE*	IncFIB, IncFIB(pLF82), IncFII, IncI1-I, Incl2(Delta)
		PS06	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, SXT, CL	115	*mcr-1, bla*$_{TEM1-B}$*, aph(6)-Id, aph(3')-Ia, aph(3")-Ib, tet(A), dfrA14, sul2, mph(A)*	*parC S80I, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFII, IncHI2, IncHI2A, IncI2(Delta)
		PS07	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	189	*mcr-1, bla*$_{TEM1-A}$ *aadA2, tet(A), dfrA12, sul1, mph(A)*	*parC S80I, gyrA D87Y, gyrA S83L*	*qacE*	Col8282, IncI1-I(Alpha), IncI2(Delta), IncX1, p0111

		PS08	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	189	*mcr-1, bla*$_{\text{TEM1-A}}$*, aadA2, tet(A), dfrA12, sul1, mph(A)*	*parC S80I, gyrA D87Y, gyrA S83L*	*qacE*	Col8282, IncI1-I(Alpha), IncI2(Delta), IncX1, p0111
		PS09	**Chicken**	NA, OFX, CIP, TE, DO, CL	117	*mcr-1, aph(6)-Id, aph(3')-Ia, aph(3')-Ib, tet(B), sul2*	*parC E84K, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFIC, IncI2(Delta)
		PS10	**Chicken**	NA, OFX, CIP, TE, SXT, CL	770	*mcr-1, aadA1, tet(A), dfrA1, sul3*	*parC* S80I, *gyrA* D87N, *gyrA* S83L	*sitABCD, qacL, qacE*	Col156, Col8282, ColpVC, IncB/O/K/Z, IncFIB), IncFII, Incl2(Delta), p0111
		PS11	**Rooster**	AMX, AMC, NA, OFX, CIP, TE, SXT, CL	1485	*mcr-1, bla*$_{\text{TEM1-B}}$*, aph(3")-Ib, aph(6)-Id, tet(A), dfrA14, sul2, mph(A)*	*parC* S80I, *gyrA* D87N, *gyrA* S83L	*sitABCD*	ColpVC, IncFIA, IncFIB, IncFIC, IncI2(Delta), p0111
		PS12	**Duck**	NA, OFX, CIP, SXT, CL	3941	*mcr-1, aadA1, aadA2b, aph(3')-Ia, sul3, cmlA1*	*parC* S80I, *gyrA* D87N, *gyrA* S83L	*sitABCD, qacL*	IncFIA, IncFIB, IncI2(Delta)
		PS13	**Drinking water**	AMX, AMC, NA, OFX, CIP, TE, DO, CL	23	*mcr-1, bla*$_{\text{TEM-1B}}$*, tet(A*	*parC S80I, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIA, IncFIB, IncFIC, IncI1-I(Alpha), IncI2(Delta)
		PS14	**Drinking water**	AMX, AMC, NA, OFX, CIP, TE, SXT, CL	354	*mcr-1, bla*$_{\text{TEM-1B}}$*, aadA1, aadA2b, aph(3')-Ia, tet(A), dfrA14, sul2, sul3, mph(A), cmlA1*	*parC S80I, parC E84G, gyrA D87N, gyrA S83L, parE I355T*	/	IncHI2, IncHI2A, IncI2(Delta)

		PS15	**Wastewater**	TE, DO, SXT, CL	453	*mcr-1, tet(B)*	/	*sitABCD*	IncI1-I(Alpha), IncI2(Delta)
		PS16	**Wastewater**	AMX, AMC, NA, OFX, CIP, TE, DO, CL	162	*mcr-1,* $bla_{\text{TEM-1B}}$, *tet(A)*	*parC* S80I, *parC* E84A, *gyrA* D87N, *gyrA* S83L	*sitABCD*	IncFIB, IncFIC, IncI2(Delta), p0111
		PS17	**Wall swab**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	189	*mcr-1,* $bla_{\text{TEM1-A}}$, *aadA2, tet(A), dfrA12, sul1, mph(A)*	*parC* S80I, *gyrA* D87Y, *gyrA* S83L	*qacE*	Col8282, IncI1-I, IncI2(Delta), IncX1, p0111
		PS18	**Soil**	AMX, AMC, NA, OFX, CIP, TE, DO, CL	162	*mcr-1,* $bla_{\text{TEM1-B}}$, *tet(A)*	*parC S80I, parC E84A, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFIC, IncI2(Delta), p0111
	3rd	PS19	**Drinking water**	AMX, AMC, NA, OFX, CIP, TE, DO, CN, SXT, CL	297	*mcr-1,* $bla_{\text{TEM1-B}}$, *aph(3')-Ia, tet(A), dfrA14, sul3, mph(A)*	*parC* S80I, *gyrA* D87N, *gyrA* S83L	*sitABCD, qacL*	IncI2(Delta), p0111
		PS20	**Soil**	AMX, AMC, NA, OFX, CIP, TE, DO, CN, SXT, CL	93	*mcr-1,* $bla_{\text{TEM-1B}}$, *aadA1, aph(3")-Ib, aph(6)-Id, qnrS1, tet(A), dfrA1, sul1, sul2, mph(A), mph(B)*	*parC* S80I, *gyrA* D87N, *gyrA* S83L	*sitABCD, qacE*	IncFI1, IncFIB, IncFIC, IncHI2, IncI2(Delta), IncQ1
B	**1st**	PS21	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	7518	*mcr-1,* $bla_{\text{TEM-1B}}$, *aph(6)-Id, aph(3')-Ia, aph(3'')-Ib, tet(A), dfrA14, sul2, mph(A)*	*parC S80I, parE S458A, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFII, IncI2(Delta), IncQ1, IncR

		PS22	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, CL	7139	*mcr-1*	*parC S80I, gyrA D87N, gyrA S83L*	/	ColpEC648, IncI1-I(Alpha), IncI2(Delta)
		PS23	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO	7139	*mcr-1,* $bla_{\text{TEM-1B}}$*, aph(3')-Ia, tet(A)*	*parC* S80I, *gyrA* D87N, *gyrA* S83L	/	ColpEC648, IncI1-I, IncI2(Delta), p0111
	2nd	PS27	**Chicken**	NA, OFX, CIP, SXT, CL	155	*mcr-1, aadA1, aadA2, tet(M), dfrA12, sul2, cmlA1, floR*	*parC* S80I, *gyrA* D87N, *gyrA* S83L *parE* S458A	*qacL*	ColpVC, IncFIB, IncI1-I, IncI2(Delta)
	3rd	PS32	**Soil**	AMX, AMC, NA, OFX, CIP, TE, DO, CN, SXT	3941	*mcr-1,* $bla_{\text{TEM-1B}}$*, aadA1, aadA2b, aph(3')-Ia, tet(A), dfrA14, sul3, mph(A), cmlA1*	*parC* S80I, *gyrA* D87N, *gyrA* S83L	*sitABCD, qacL*	IncFIA, IncFIB, IncI1-I, IncI2(Delta)
C	**1st**	PS35	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	744	*mcr-1, tet(B), dfrA14, mph(A), catA1*	*parC S80I, parC A56T, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIA, IncFIB, IncFIC, IncI2(Delta)
		PS36	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, CL	162	*mcr-1,* $bla_{\text{TEM-1B}}$*, tet(A)*	*parC* S80I, *gyrA* D87N, *gyrA* S83L, *parC* E84A	*sitABCD*	IncFIB, IncFIC, IncI2(Delta)
		PS37	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	1011	*mcr-1,* $bla_{\text{TEM-1B}}$*, aadA1, aadA2b, tet(A), dfrA14, sul3, mph(A), cmlA1*	*parC S80I, gyrA D87N, gyrA S83L*	*sitABCD, qacE*	Col156, IncFIB, IncFIC, IncI1-I(Alpha), IncI2(Delta)

		PS38	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, CN, SXT, CL	162	*mcr-1, bla*$_{\text{TEM-1B}}$*, aadA1, aadA2, aph(3')-Ia, tet(A), dfrA14, sul3, cmlA1*	*parC S80I, parC E84A, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFIC, IncI2(Delta), p0111
	2nd	PS39	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	354	*mcr-1, bla*$_{\text{TEM-1B}}$*, aph(6)-Id, aph(3'')-Ib, tet(A), dfrA7, sul1, sul2*	*parC S80I, parC E84G, gyrA D87N, gyrA S83L parE I355T*	*qacE*	IncI2(Delta), IncQ1
		PS40	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, CL	162	*mcr-1, bla*$_{\text{TEM-1B}}$*, tet(A)*	*parC S80I, parC E84A, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFIC, IncI2(Delta)
		PS41	**Chicken**	NA, OFX, CIP, TE, DO, SXT, CL	224	*mcr-1, aadA1, aadA2, tet(A), dfrA12, sul3, cmlA1*	*parC* S80I, *gyrA* D87N, *gyrA* S83L *parE* S458A	*qacL*	IncI2(Delta), IncX4
		PS42	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, CL	162	*mcr-1, bla*$_{\text{TEM-1B}}$*, tet(A)*	*parC S80I, parC E84A, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFIC, IncI1-I(Alpha), IncI2(Delta)
	3rd	S260	**Soil**	AMX, AMC, NA, OFX, CIP, TE, DO, CN, SXT, CL	226	*mcr-1, bla*$_{\text{TEM-1B}}$*, blaSHV-1 aadA2, aph(3')-Ia, tet(A), tet(D), dfrA12, sul1, sul2, mph(A)*	*parC S80I, gyrA D87N, gyrA S83L*	*qacE*	IncFIB, IncFII, IncI2(Delta)

		S261	**Soil**	NA, OFX, CIP, TE, DO, CL	11956	*mcr-1*	*parC* S80I, *gyrA* D87N, *gyrA* S83L	/	Col156, IncFIB, IncI2(Delta), IncY
	4th	PS45	**Chicken**	NA, TE, DO, CL	616	*mcr-1, tet(A)*	*gyrA* S83L	*sitABCD*	IncFIB, IncFIB(pLF82), IncFIC, IncI1-I, IncI2(Delta)
		PS46	**Chicken**	NA, TE, DO, CL	616	*mcr-1, tet(A)*	*gyrA S83L*	*sitABCD*	IncFIB, IncFIC, IncI1-I(Alpha), IncI2(Delta)
		PS47	**Chicken**	NA, TE, DO, CL	616	*mcr-1, tet(A)*	*gyrA S83L*	*sitABCD*	IncFIB, IncFIC, IncI1-I(Alpha), IncI2(Delta)
		PS48	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, SXT, CL	616	*mcr-1,* $bla_{\text{TEM-1B}}$*, tet(A), dfrA14, sul2, mph(A), catA1*	*gyrA S83L*	*sitABCD*	IncFIB, IncFIC, IncI1-I(Alpha), IncI2(Delta), IncX1, p0111
		PS49	**Chicken**	NA, OFX, CIP, TE, DO, CL	616	*mcr-1, tet(A)*	*gyrA S83L*	*sitABCD*	IncFIB, IncFIC, IncI1-I(Alpha), IncI2(Delta)
		PS50	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	101	*mcr-1,* $bla_{\text{TEM-1B}}$*, tet(A), dfrA14, sul2, mph(A), catA1*	*parC* S80I, *gyrA* D87N, *gyrA* S83L	*sitABCD*	IncFIB, IncFIC, IncI2(Delta), IncX1, p0111
		PS51	**Drinking water**	NA, TE, DO, CL	616	*mcr-1, tet(A)*	*gyrA* S83L	*sitABCD*	IncFIB, IncFIB(pLF82), IncFIC, IncI1-I, IncI2(Delta)

		PS52	**Drinking water**	MX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	101	*mcr-1, bla*$_{\text{TEM-1B}}$*, tet(A), dfrA14, sul2, mph(A), catA1*	*parC* S80I, *gyrA* D87N, *gyrA* S83L	*sitABCD*	IncFIB, IncFIC, IncI2(Delta), IncX1, p0111
		PS53	**Wall swab**	MX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	101	*mcr-1, bla*$_{\text{TEM-1B}}$*, tet(A), dfrA14, sul2, mph(A), catA1*	*parC S80I, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFIC, IncI2(Delta), IncX1, p0111
		PS54	**Wall swab**	NA, TE, DO, CL	616	*mcr-1, tet(A)*	*gyrA* S83L	*sitABCD*	IncFIB, IncFIB(pLF82), IncFIC, IncI1-I, IncI2(Delta)
		PS55	**Soil**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	101	*mcr-1, bla*$_{\text{TEM-1B}}$*, tet(A), dfrA14, sul2, mph(A), catA1*	*parC* S80I, *gyrA* D87N, *gyrA* S83L	*sitABCD*	IncFIB, IncFIC, IncI2(Delta), IncX1, p0111
D	**1st**	S09	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	648	*mcr-1, bla*$_{\text{TEM-1B}}$*, tet(A), dfrA14, sul2, mph(A)*	*parC S80I, gyrA D87N, gyrA S83L*	/	ColpVC, IncFIB, IncFIC, IncI1-I(Alpha), IncI2(Delta)
		S10	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	1640	*mcr-1, bla*$_{\text{TEM-1B}}$*, aph(6)-Id, aph(3'')-Ib, tet(A), sul2, catA1*	*parC S80I, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFII, IncI2(Delta), IncQ1
		S11	**Chicken**	NA, OFX, CIP, TE, DO, CL	93	*mcr-1, tet(A)*	*parC* S80I, *gyrA* D87N, *gyrA* S83L	*sitABCD*	IncFIB, IncI2(Delta)
		S12	**Chicken**	NA, OFX, CIP, TE, DO, CL	93	*mcr-1, tet(A)*	*parC S80I, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFII, IncI2(Delta)
		S13	**Chicken**	NA, OFX, CIP, TE, DO, CL	93	*mcr-1, tet(A)*	*parC S80I, gyrA D87N, gyrA S83L*	*sitABCD*	IncFIB, IncFII, IncI2(Delta)

		S14	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	58	*mcr-1, bla*$_{\text{TEM-1B}}$*, tet(A), dfrA14, sul2, mph(A)*	*parC S80I,* *gyrA D87Y, gyrA S83L*	*sitABCD*	Col8282, ColpVC, IncFIA, IncFIB, IncFIC, IncI1-I(Alpha), IncI2(Delta)
E	**1st**	S204	**Chicken**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	8492	*mcr-1, bla*$_{\text{TEM-1B}}$*, aph(3')-Ia, tet(A), dfrA14, sul3, mph(A)*	*parC* S80I, *parE* S458A, *gyrA* D87N, *gyrA* S83L	*qacL*	IncFIB, IncFIC, IncI1-I, IncI2(Delta), p0111
		S209	**Soil**	AMX, AMC, NA, OFX, CIP, TE, DO, SXT, CL	8492	*mcr-1, bla*$_{\text{TEM-1B}}$*, aph(3')-Ia, tet(A), dfrA14, sul3, mph(A)*	*parC* S80I, *parE* S458A, *gyrA* D87N, *gyrA* S83L	*qacL*	IncFIB, IncFIC, IncI1-I, IncI2(Delta), p0111

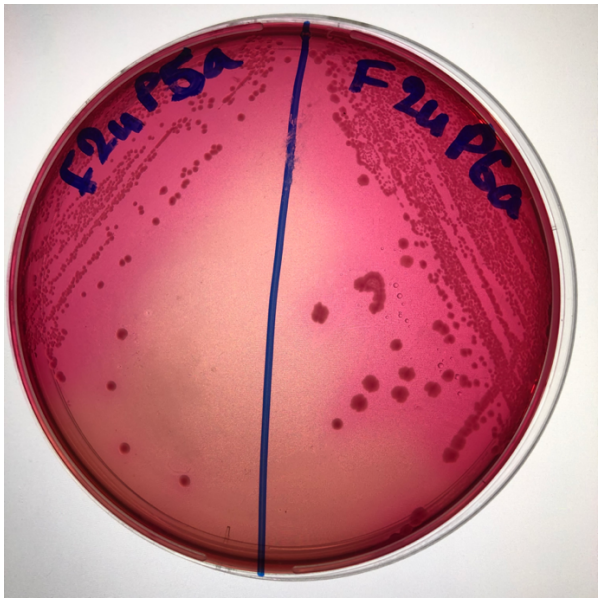

Figure S1: Screening of resistant *Escherichia coli* on selective MacConkey media

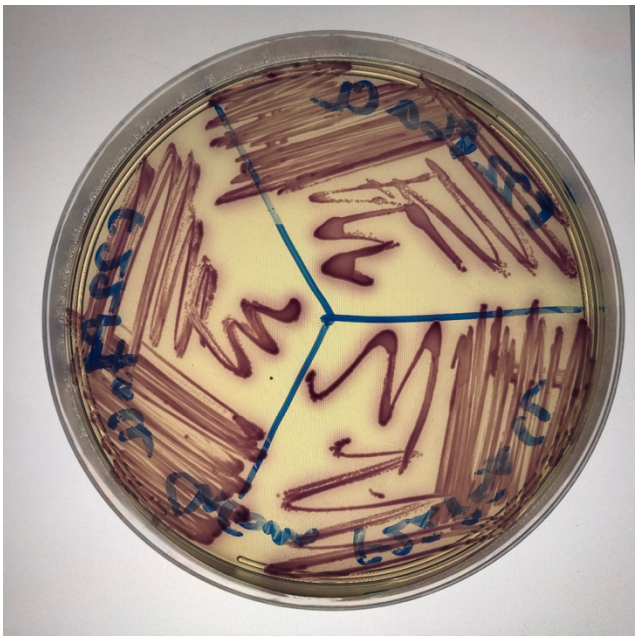

Figure S2: Presumptive identification of *Escherichia coli* on CHROMagar media

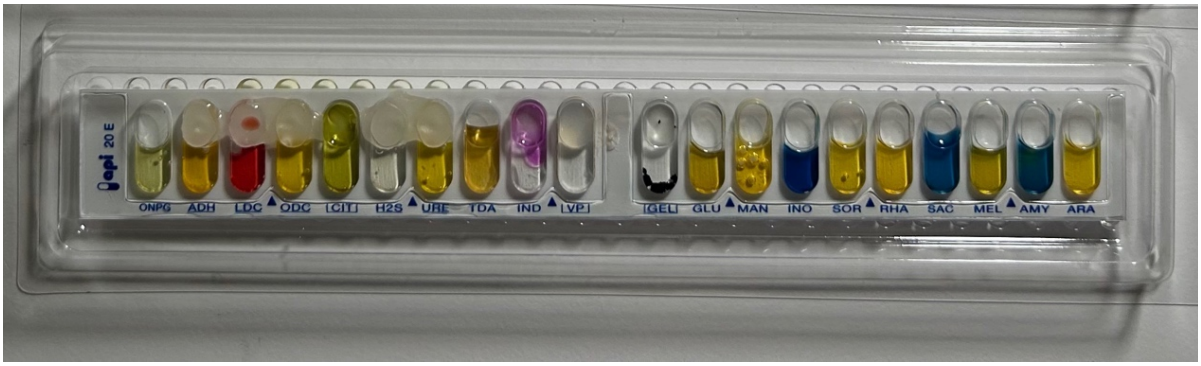

Figure S3: Presumptive identification of *Escherichia coli* with the API 20 E gallery

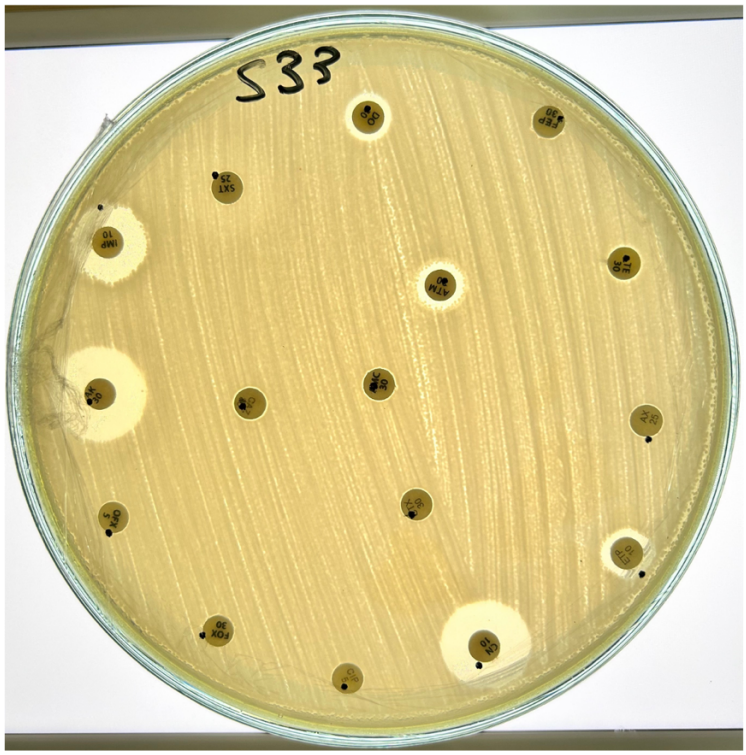

Figure S4: Determination of susceptibility profiles with the disc diffusion method

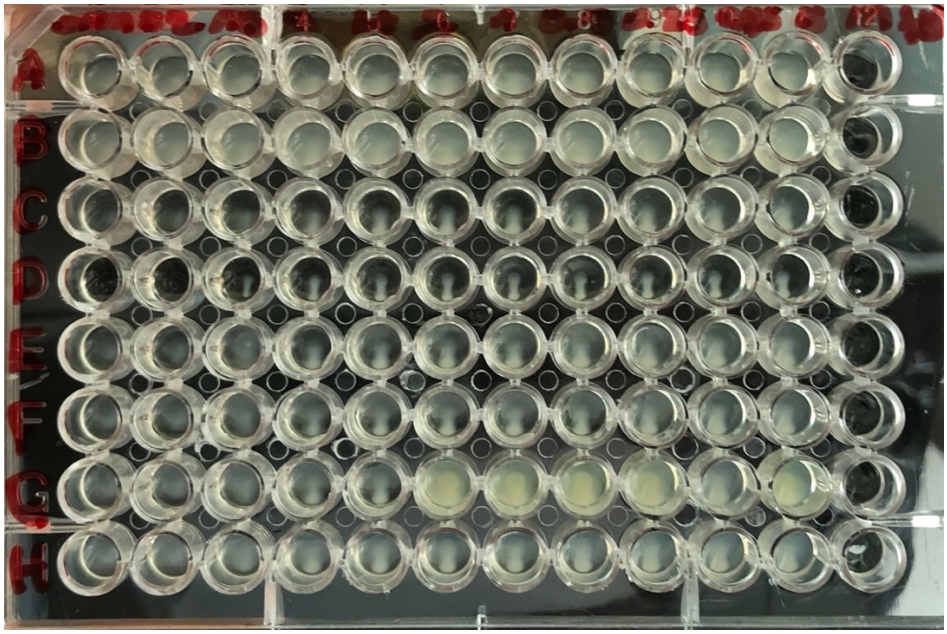

Figure S5: Determination of colistin minimum inhibitory concentration with the broth microdilution method

Scientific production

Article:

1. Kirat, H., Rahab, H., Chekroud, Z., Abbassi, M. S., Basher, N. S., Ibrahim, N. A., & Touati, A. (2025). Fecal carriage of resistant Escherichia coli in livestock in Algeria: Emergence of NDM and OXA-181. *BMC Microbiology*, *25*(1), 473. https://doi.org/10.1186/s12866-025-04174-2
2. Sassi A, Basher NS, Kirat H, et al. The Role of the Environment (Water, Air, Soil) in the Emergence and Dissemination of Antimicrobial Resistance: A One Health Perspective. Antibiotics 2025;14(8):764; doi: 10.3390/antibiotics14080764.
3. Touati A, Ibrahim NA, Mairi A, et al. One Health at Risk: Plasmid-Mediated Spread of mcr-1 Across Clinical, Agricultural, and Environmental Ecosystems. Antibiotics 2025;14(5):506; doi: 10.3390/antibiotics14050506.

International communications:

1. Kirat, H., Chekroud, Z., Bounneche, M. H., Rahab, H. (2021, December). Détection D'*Escherichia coli* productrice de β-Lactamases à spectre étendu chez des bovins d'engraissement dansl a Région de Guelma. 1st International Seminar on Pollution, Health, Environment and Bio-monitoring, Skikda, Algeria.
2. Kirat, H., Chekroud, Z., Rahab, H. (2022a, March). Première détection de souches d'*Escherichia coli* et de *Klebsiella pneumoniae* productrice de β-lactamase à spectre étendu chez des bovins d'engraissement dans la région de Guelma en Algérie. 1st international days of natural and life sciences, Ouargla, Algeria.

National communications:

1. Kirat, H., Chekroud, Z., Rahab, H. (2022b, October). Première détection des souches d'*Escherichia coli* productrices de β-lactamases à spectre étendu chez l'aviaire dans a wilaya de Guelma. Le premier séminaire national sur la biotechnologie et la biodiversité microbienne, Khenchela, Algeria.
2. Kirat, H., Chekroud, Z., Rahab, H. (2022c, December). Dessimination of extended spectrum β-lactamase and multidrug resistant *Escherichia coli* among fattening cattle in Guelma. Premier séminaire national sur la zootechnie, Souk Ahras, Algeria.

3. Kirat, H., Chekroud, Z., Rahab, H. (2022d, December). Fecal carriage of extended spectrum β-lactamases producing and multidrug resistant *Escherichia coli* in cattle in two fattening farms in Guelma. Journée nationale sur l'antibiorésistance et environnement, Constantine, Algeria.
4. Kirat, H., Chekroud., Z, Rahab, H., Abbassi, M. S., Touati, A. Dissemination of extended spectrum β-lactamase-producing and multidrug resistant *Escherichia coli* among Cattle in Guelma. The first national congress on phages and other innovative solutions to fight antibiotic resistance, Constantine, Algeria

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Fecal carriage of resistant *Escherichia coli* in livestock in Algeria: emergence of NDM and OXA-181

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Abstract

Introduction The spread of third-generation cephalosporin (3GC)-resistant *Escherichia coli* in food-producing animals poses a significant threat to public health, with limited data from cattle and sheep in Algeria. This study investigated the prevalence of 3GC-resistant *E. coli* in cattle and sheep in Guelma, northeast Algeria.

Methodology Two hundred eighty-five fecal samples were collected from cattle (*n*=145) and sheep (*n*=140) on 28 farms. Samples were screened for 3GC-resistant *E. coli*. Antibiotic susceptibility was tested, and ESBL and carbapenemase production were evaluated using double disc and EDTA tests. PCR identified resistance and integron genes.

Results Twenty-seven cefotaxime-resistant *E. coli* isolates were detected in 17% of bovine and 1% of ovine samples, spanning 43% of the farms. Multidrug resistance was observed in 85% of isolates, with high resistance to β-lactams, tetracyclines, fluoroquinolones, and trimethoprim-sulfamethoxazole. The following beta-lactamase genes were detected: $bla_{\text{CTX-M}}$ (74%), bla_{CMY} (44%), $bla_{\text{NDM-1}}$ (37%), and $bla_{\text{OXA-181}}$ (4%) were identified. Class 1 integrons were also detected in ten isolates.

Conclusions These findings emphasize the presence of ESBL-, AmpC-, and carbapenemase-producing *E. coli* among Algerian livestock, highlighting the need for comprehensive monitoring and control to manage the spread of these resistant bacteria.

Keywords Algeria, Cattle, Sheep, 3GC-resistant *E. coli*, ESBL, Carbapenemases

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Review

The Role of the Environment (Water, Air, Soil) in the Emergence and Dissemination of Antimicrobial Resistance: A One Health Perspective

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Abstract

Antimicrobial resistance (AMR) has emerged as a planetary health emergency, driven not only by the clinical misuse of antibiotics but also by diverse environmental dissemination pathways. This review critically examines the role of environmental compartments—water, soil, and air—as dynamic reservoirs and transmission routes for antibiotic-resistant bacteria (ARB) and resistance genes (ARGs). Recent metagenomic, epidemiological, and mechanistic evidence demonstrates that anthropogenic pressures—including pharmaceutical effluents, agricultural runoff, untreated sewage, and airborne emissions—amplify resistance evolution and interspecies gene transfer via horizontal gene transfer mechanisms, biofilms, and mobile genetic elements. Importantly, it is not only highly polluted rivers such as the Ganges that contribute to the spread of AMR; even low concentrations of antibiotics and their metabolites, formed during or after treatment, can significantly promote the selection and dissemination of resistance. Environmental hotspots such as European agricultural soils and airborne particulate zones near wastewater treatment plants further illustrate the complexity and global scope of pollution-driven AMR. The synergistic roles of co-selective agents, including heavy metals, disinfectants, and microplastics, are highlighted for their impact in exacerbating resistance gene propagation across ecological and geographical boundaries. The efficacy and limitations of current mitigation strategies, including advanced wastewater treatments, thermophilic composting, biosensor-based surveillance, and emerging regulatory frameworks, are evaluated. By integrating a One Health perspective, this review underscores the imperative of including environmental considerations in global AMR containment policies and proposes a multidisciplinary roadmap to mitigate resistance spread across interconnected human, animal, and environmental domains.

Keywords: antimicrobial resistance; antibiotic-resistant bacteria; environmental pollution; One Health; horizontal gene transfer; metagenomics; waterborne pathogens

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Review

One Health at Risk: Plasmid-Mediated Spread of *mcr-1* Across Clinical, Agricultural, and Environmental Ecosystems

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Abstract: The global dissemination of plasmid-mediated *mcr* genes, which confer resistance to the last-resort antibiotic colistin, represents a critical public health challenge driven by the interplay of clinical, agricultural, and environmental factors. This review examines the genetic and ecological dynamics of *mcr*-bearing plasmids, focusing on their role in disseminating colistin resistance across diverse bacterial hosts and ecosystems. Key plasmid families demonstrate distinct evolutionary strategies, including IncI2, IncHI2, and IncX4. IncI2 plasmids favor stability in livestock and clinical settings. IncHI2 plasmids, on the other hand, leverage transposons to co-select for multidrug resistance, while IncX4 plasmids achieve global dissemination through streamlined, conjugation-efficient architectures. The pervasive spread of *mcr* genes is exacerbated by their integration into chromosomes via mobile genetic elements and co-selection with resistance to other antibiotic classes, amplifying multidrug-resistant phenotypes. Environmental reservoirs, food chains, and anthropogenic practices further facilitate cross-niche transmission, underscoring the interconnectedness of resistance under the One Health framework. Addressing this crisis requires coordinated strategies, including reducing colistin misuse in agriculture, enhancing surveillance of high-risk plasmid types, and fostering international collaboration to preserve antimicrobial efficacy and mitigate the threat of untreatable infections.

Keywords: plasmid-mediated resistance; *mcr-1* gene; colistin resistance; IncI2 plasmids; IncHI2 plasmids; IncX4 plasmids; one health

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1. Introduction

Colistin, also known as polymyxin E, was first discovered in the 1940s and introduced for clinical use in the 1950s, but its early promise was curtailed by its significant nephrotoxicity and neurotoxicity [1]. With the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacteria, colistin has been globally re-introduced as a last-resort antibiotic to treat infections caused by pathogens such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* [2,3]. Its use has

Abstract

Antibiotic resistance is a global health concern, threatening both human and veterinary medicine, with livestock acting as a major reservoir for the dissemination and the persistence of resistant bacteria particularly third-generation cephalosporin-resistant (3GC-R) and colistin-resistant (CL-R) Enterobacterales. This thesis investigated the dissemination of 3GC-R and CL-R *Escherichia coli* among poultry, bovine and ovine farms in Guelma city, northeastern Algeria. A longitudinal surveillance was further performed in order to assess the persistence of CL-R isolates in poultry farms over production cycles. A total of 919 animal and environmental samples were collected from 38 farms in Guelma. Samples were screened for 3GC-R and CL-R *E. coli* in selective medias. The screened isolates were identified then subjected to susceptibility tests, including the disc diffusion test, the determination of colistin minimum inhibitory concentration, and the phenotypic detection of ESBL and carbapenemase production. Afterwards, standard PCR was performed to the 3GC-R isolates to investigate the presence of antibiotic resistance genes and integrons, whereas the CL-R were submitted to whole genome sequencing. Overall, 112 isolates were collected form 21 farms, including 58 3GC-R and 54 CL-R *E. coli* strains. The 3GC-R isolates were detected in poultry, bovine and ovine farms, while CL-R strains were only found in poultry farms. Phenotypic tests revealed that the majority of the isolates were multidrug resistant and displayed high resistance rates against β-lactams, tetracyclines, fluoroquinolones and sulfonamides. The molecular analysis identified critical resistance genes including *mcr-1*, $bla_{\text{CTX-M}}$, bla_{NDM}, $bla_{\text{OXA-181}}$, bla_{CMY} and bla_{DHA}. The MSLT analysis identified 28 different Sequence Types (STs), mainly ST162 and ST93, and provided evidences on the clonal dissemination and the potential persistence of several STs within the farms. These findings underscore the great role of livestock as a reservoir for resistant bacteria and their genes and emphasize the urgent need for targeted strategies within a “One Health” framework to mitigate the spread of these isolates and preserve the efficiency of critical antimicrobial agents.

Key words: Antibiotic-resistant *E. coli*, Colistin, Third-generation cephalosporins, ESBL, Carbapenemase, AmpC, MCR, Livestock.