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Enterobacteriaceae and Antimicrobial Resistance: A Study on Dromedaries within the "One Health" Context

Presented by:

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Statement of Honour

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For everysleepless night, for everytear held back and every smile pushed through.
For believing, even in silence, even in doubt.
For rising, again and again.
This moment is not an end
It is a quiet, powerful beginnin

Summary

List of Abbreviations

API	Analytical Profile Index
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Testing
BHRe	Bactéries Hautement Résistantes émergentes (Highly Resistant Emerging Bacteria)
CLSI	Clinical and Laboratory Standards Institute
ECA	Enterobacterial Common Antigen
ESBL	Extended-Spectrum Beta-Lactamase
ExPEC	Extraintestinal Pathogenic Escherichia coli
FBIs	Foodborne Illnesses
GRE / VRE	Glycopeptide-Resistant Enterococci / Vancomycin-Resistant Enterococci
HGT	Horizontal Gene Transfer
MDR	Multidrug-Resistant
MDRB	Multidrug-Resistant Bacteria
MIC	Minimum Inhibitory Concentration
MR	Methyl Red
MRSA	Methicillin-Resistant Staphylococcus aureus
ONPG	Ortho-Nitrophenyl-β-D-Galactopyranoside
PBP	Penicillin-Binding Protein
PDR	Pan Drug-Resistant
QRDR	Quinolone Resistance-Determining Region
ROS	Reactive Oxygen Species
S-type / R type	Smooth colony type / Rough colony type
TSI	Triple Sugar Iron (Agar Medium)
UPEC	Uropathogenic Escherichia coli
VP	Voges-Proskauer
WHO	World Health Organization
WOAH	World Organisation for Animal Health

Abstract

This study investigates the presence of Enterobacteriaceae in dromedaries and assesses their antibiotic resistance profiles in the Algerian Sahara. A total of 50 fecal samples were collected and subjected to isolation, biochemical identification, and antimicrobial susceptibility testing. The results highlight a notable presence of multidrug-resistant (MDR) strains, including those producing Extended-Spectrum Beta-Lactamases (ESBLs). These findings raise concerns about the dromedary as a potential reservoir of antimicrobial resistance genes, emphasizing its role within the ―One Health‖ framework. The study underlines the importance of integrating animal surveillance into broader public health strategies to mitigate the spread of resistant bacteria.

Keywords: Enterobacteriaceae, Antimicrobial resistance, Dromedary, One Health, ESBL, Multidrug resistance, Algeria

Résumé

Cette étude porte sur la présence des entérobactéries chez les dromadaires et sur leur profil de résistance aux antibiotiques dans la région saharienne de l'Algérie. Cinquante échantillons fécaux ont été analysés par isolement, identification biochimique et antibiogramme. Les résultats révèlent une présence importante de souches multirésistantes (MDR), notamment productrices de β-lactamases à spectre étendu (ESBL). Ces résultats suggèrent que le dromadaire pourrait constituer un réservoir potentiel de gènes de résistance, renforçant la nécessité de l'intégrer dans le cadre du concept « One Health ». L'étude met en évidence l'importance d'une surveillance vétérinaire intégrée aux politiques de santé publique.

Mots-clés : Entérobactéries, Résistance aux antimicrobiens, Dromadaire, One Health, ESBL, Multirésistance, Algérie

الملخص

عينة من البراز، وفحصها 50 تم جمع .في الجمال، وتقييم مدى مقاومتها للمضادات الحيوية في منطقة الصحراء الجزائرية (E. coli) .باستخدام الزرع والعزل والتشخيص البيوكيميائي، إضافةً إلى اختبار الحساسية للمضادات الحيوية

مما يشير إلى ،(ESBL) لاكتاماز واسع الطيف-أظهرت النتائج وجود سلالات متعددة المقاومة، بما في ذلك السلالات المنتجة لإنزيم البيتا ."صحة واحدة" أن الجِمال قد تشكل خزّانًا محتملًا لبكتيريا مقاومة للمضادات الحيوية، وهو ما يُبرز أهمية هذه النتائج في إطار مفهوم .وتؤكد الدراسة أهمية دمج المقاربات البيطرية ضمن استراتيجيات الصحة العامة للحد من انتشار السلالات المقاومة

الكلمات المفتاحية:

.الجزائر، صحة واحدة ،ESBL ،تعدد المقاومة، الإشريكية القولونية، مقاومة المضادات الحيوية، الجِمال

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Introduction

Antimicrobial resistance (AMR) represents one of the most pressing global threats to both human and animal health in the 21st century. As outlined by the World Health Organization (WHO) and the World Organisation for Animal Health (WOAH), the proliferation of resistant bacterial strains—particularly among Gram-negative pathogens such as those in the Enterobacteriaceae family—poses significant challenges to clinical treatment, public health infrastructure, and sustainable livestock production. **(Alhassan & Ahmad, 2025)**

Enterobacteriaceae, a diverse group of facultative anaerobic Gram-negative bacilli, are commonly encountered in the gastrointestinal flora of humans and animals. While many species function as commensals, others such as *Escherichia coli*, *Salmonella spp.*, and *Klebsiella pneumoniae* are implicated in severe zoonotic and nosocomial infections. Their remarkable capacity to acquire and disseminate resistance determinants—via mechanisms such as plasmid-mediated conjugation, transduction, and transformation—has enabled them to persist and evolve in varied ecological niches, including food-producing animals. **(Janda & Abbott, 2021)**

The dromedary camel (*Camelus dromedarius*), a cornerstone species in agro-pastoral systems of arid and semi-arid regions, has traditionally received limited attention in AMR surveillance efforts. However, emerging evidence suggests that this species may serve as a potential reservoir of multidrug-resistant (MDR) bacteria. In Algeria‘s Saharan context, where camels are raised under extensive systems with minimal veterinary oversight, the misuse or empirical administration of antimicrobials—often without prior antibiogram testing—heightens the risk of resistance emergence and dissemination.

Within this framework, our study sought to address a significant knowledge gap by investigating the prevalence and antimicrobial susceptibility profiles of Enterobacteriaceae isolated from dromedary fecal matter. Conducted in the Ouargla region of southern Algeria, this work was developed in alignment with the "One Health" concept, which recognizes the interconnectedness of human, animal, and environmental health. Specifically, we aimed to (1) isolate and biochemically identify Enterobacteriaceae from camel feces, (2) assess their resistance profiles through standardized antibiogram techniques, and (3) evaluate the potential epidemiological role of dromedaries as vectors or reservoirs of antimicrobial resistance. **(Sara et al., 2021)**

The findings of this study not only contribute to the broader surveillance of AMR in non-conventional livestock but also underscore the importance of integrating camel health into national and regional public health strategies. By doing so, we can better anticipate and mitigate the risks posed by zoonotic transmission and environmental contamination, thereby reinforcing the foundational pillars of One Health in North Africa and beyond. **(Bilal et al., 2025)**

Chapter I: Enterobacteriaceae

I. General Considerations on Enterobacteriaceae

Foodborne illnesses (FBIs) remain a major global public health concern. These infections occur following the consumption of foodstuffs or beverages contaminated with pathogenic agents such as bacteria, viruses, or parasites. Once ingested, these microorganisms can proliferate within the host organism, establish colonization, invade tissues, and/or produce toxins that are responsible for a wide range of gastrointestinal symptoms, some of which may be severe **(FAO/WHO, 2023; EFSA, 2022).**

Pathogenic bacteria, particularly members of the *Enterobacteriaceae* family, are frequently associated with food products of animal origin (meat, dairy products, eggs), thereby compromising their microbiological safety. These microorganisms are implicated in various clinical manifestations ranging from mild diarrhea to septicemic syndromes, depending on the specific bacterial species involved, the infectious dose, and the host‘s susceptibility **(Silva et al., 2021; Chlebicz & Śliżewska, 2020).**

I.1. Definition of Enterobacteriaceae

Enterobacteriaceae constitute a group of Gram-negative bacteria belonging to the order *Enterobacterales*. These are non-spore-forming bacilli, generally motile due to peritrichous flagella; however, certain genera such as *Klebsiella* and *Shigella* are non-motile. They are facultative anaerobes and readily grow on conventional nutrient media **(Chandran et al., 2022).**

Biochemically, these organisms are oxidase-negative and catalase-positive (with rare exceptions), capable of reducing nitrates to nitrites, and they ferment glucose with species-dependent production of gases or organic acids **(Lopez et al., 2021).**

Their cell wall is composed of lipopolysaccharides (LPS), with the O-polysaccharide portion being antigenic. When present, flagella bear H antigens, while certain species express capsular K antigens. These antigenic components are critical for serological classification and are closely linked to the pathogenicity of specific strains **(Kumar et al., 2023).**

Historically, the genus *Enterobacter* was first proposed by Otto Rahn in 1937 to group bacteria exhibiting similar phenotypic traits. Over time, the family *Enterobacteriaceae*

underwent substantial expansion, notably through the taxonomic contributions of Don Brenner and Patrick A.D. Grimont. Initially, this family encompassed major genera such as *Escherichia*, *Salmonella*, *Klebsiella*, *Proteus*, *Serratia*, and *Shigella* **(Adeolu et al., 2016).**

I.1.1. Recent Taxonomic Evolution

Advancements in molecular biology and comparative phylogenetics—particularly through 16S rRNA sequencing and whole-genome analyses—have led to a major revision of the taxonomy of Enterobacteriaceae. As a result, the former family has been reorganized, and the order *Enterobacterales* is now subdivided into several distinct families:

- **Enterobacteriaceae** (*Escherichia, Salmonella, Klebsiella,* etc.)
- **Morganellaceae** (*Proteus, Morganella, Providencia*)
- **Yersiniaceae** (*Yersinia, Serratia*)
- **Erwiniaceae**, **Hafniaceae**, **Pectobacteriaceae**, among others **(GTDB, 2023).**

I.1.2. Distribution and Ecology

Enterobacteriaceae are ubiquitous microorganisms. They are commonly found in fecal matter, wastewater, soil, and healthcare environments. They constitute more than 10% of the microbial flora in the distal intestine of humans and warm-blooded animals **(García et al., 2021)**. Certain species are also capable of colonizing the oral cavity, upper respiratory tract, and genitourinary system.

I.1.3. Diversity and Differential Characteristics

The Enterobacteriaceae family currently encompasses over 200 species distributed among more than 40 genera. Differentiation among these genera is based on precise phenotypic criteria, including the ability to ferment specific carbohydrates, production of hydrogen sulfide (H_2S), enzymatic activity (e.g., indole production, urease, citrate utilization), as well as serotyping (**Kumar et al., 2023**).

I.1.4. Phylogenetic Classification

- According to the most recent genomic data, the Enterobacteriaceae are classified as follows:
- **Domain**: *Bacteria*
- **Phylum**: *Pseudomonadota* (formerly *Proteobacteria*)
- **Class**: *Gammaproteobacteria*
- **Order**: *Enterobacterales*
- **Families**: *Enterobacteriaceae*, *Morganellaceae*, *Yersiniaceae*, among others (**GTDB Release 214, 2024; Parte et al., 2023**).

I.2. Bacteriological Characteristics of Enterobacteriaceae

I.2.1. Morphological Characteristics

Enterobacteriaceae are Gram-negative bacilli, rod-shaped, measuring between 0.5 and 5 micrometers in length and 0.2 to 1 micrometer in width (**Willey et al., 2017; Madigan et al., 2018**).

The genus *Proteus* exhibits a wide morphological diversity, ranging from elongated filamentous forms to short straight bacilli. Most species are motile, owing to the presence of peritrichous flagella. Others, such as *Klebsiella*, *Shigella*, and *Yersinia pestis*, are non-motile (**Murray et al., 2016**).

Klebsiella species are encapsulated. Common fimbriae or pili serve as adhesion factors for most human pathogenic species. These bacteria also express a conserved surface antigen known as the Enterobacterial Common Antigen (ECA or Kunin antigen) (**Bakhoum, 2004**).

I.2.2. Cultural Characteristics

Generally, Enterobacteriaceae can be cultivated on basic nutrient-rich media such as standard agar containing peptones, meat extracts, and carbohydrates. However, certain selective and differential media are specifically designed to promote the growth of intestinal bacteria while inhibiting that of others. For instance, MacConkey agar contains crystal violet

and bile salts, which inhibit the growth of most Gram-positive bacteria while selectively favoring the growth of Enterobacteriaceae (**Murray et al., 2016; Willey et al., 2017**).

Enterobacteria proliferate rapidly; for most species, colonies form after 18–24 hours of incubation at 35–37°C. These colonies are convex, round, with well-defined edges, a smooth and shiny surface, and are referred to as ―smooth‖ or S-type colonies. When subcultured into broth, S-phase colonies produce uniform turbidity throughout the entire column of the tube.

After repeated subculturing, S-type strains may transition to R-type (―rough‖) colonies, which are dry, flat, irregularly edged, and exhibit a dull appearance. These rough-phase colonies produce a granular culture upon agitation and tend to spontaneously form sedimenting agglutinates. Moreover, they may exhibit auto-agglutination when suspended in saline solution (**Joly and Reynaud, 2002**).

Capsule producing bacterial colonies, such as those of *Klebsiella pneumoniae*, appear mucoid and are larger than typical colonies, sometimes reaching up to 10 mm in diameter. These colonies exhibit a gelatinous consistency. *Proteus vulgaris* and *Proteus mirabilis* are particularly motile species, a characteristic that often results in swarming across the surface of solid media. This swarming occurs in successive waves and can cover the entire surface of the medium within 24 hours (**Bakhoum, 2004; Pilet et al., 1979**).

Nevertheless, some Enterobacteriaceae exhibit poor growth, resulting in very small colonies, referred to as "pinpoint colonies." This often reflects a requirement for one or more growth factors. Other bacteria demonstrate slow growth and require several days of incubation to produce normally sized colonies; this is notably the case for certain species within the *Shigella* and *Yersinia* genera. For the latter, for instance, incubation at 37 °C for at least 48 hours is necessary (**Bidet & Bingen, 2007**).

Enterobacteriaceae are chemoorganotrophic, and some strains are prototrophic. They are capable of synthesizing all the essential components required for their survival and growth from a single carbon source (such as a sugar) and an energy source (electrons) (**Avril et al., 2000)**.

I.2.3. Biochemical Characteristics

The identification characteristics of Enterobacteriaceae are primarily *biochemical* in nature. These include tests that assess protein metabolism (such as urease activity, indole

production, and tryptophan degradation), sugar fermentation (e.g., glucose, lactose, sucrose), the ability to utilize citrate as the sole carbon source, the production of acetoin (Voges-Proskauer reaction), the presence of specific enzymes (decarboxylases, deaminases), the ability to reduce nitrates to nitrites, and the production of hydrogen sulfide or gas formation (**Murray et al., 2016**).

Identification is performed in test tubes to ensure both optimal bacterial growth and accurate observation of biochemical reactions. Notably, innovative approaches to this method have emerged, such as the development of the API 20E system—the first identification strip designed specifically for enteric bacteria—and the introduction of automated systems like the MINI API (**Bébéar et al., 2006).** In the laboratory, Enterobacteriaceae are frequently identified based on their biochemical profiles using targeted biochemical assays.

The differential biochemical characteristics of selected Enterobacteriaceae are presented in Table 01.

Table 01: Key biochemical characteristics of selected Enterobacteriaceae (**Gadou, 2019**).

Species	Glucose (Fermentation)	Gaz	Motility	Urease	H_2S	Lysine Decarboxylase	Indole
Escherichia coli	+	+	+	–	–	+	+
Salmonella enterica	+	+	+	–	+	+	–/+
Klebsiella pneumoniae	+	+	–	+	–	+	–
Shigella sonnei	+	±	–	–	–	–	–
Proteus mirabilis	+	+	+	+	+	–	–
Enterobacter cloacae	+	+	+	+	–	+	–/+
Serratia marcescens	+	+	+	–	–	–	–
Citrobacter freundii	+	+	+	+	+	–	–/+
Morganella morganii	+	+	+	+	–	–	+
Yersinia enterocolitica	+	±	±	+	–	–	

Legend : +: positive; –: negative; ±: variable;H_2S: hydrogen sulfide production; Indole: indole test;Gas: gas production from glucose.

I.2.4. Antigenic Characteristics

Strains belonging to the same species or genus can be classified into serotypes based on their diverse antigenic profiles. Certain pathogenic Enterobacteriaceae such as *Salmonella*, *Shigella*, and *Escherichia coli* hold significant epidemiological value in serotype determination.

Antigens can be identified using various techniques, the most common being the slide agglutination method with specific antisera: the presence of agglutination indicates a match between the applied antiserum and an antigen expressed by the tested strain (**Avril et al., 2000**).

Several types of antigens are distinguished:

1. Common Antigen

All Enterobacteriaceae, with the exception of certain *Erwinia* species, possess a shared antigen known as the "Kunin antigen." It has taxonomic relevance. Typically, this antigen exists in a haptenic, non-immunogenic form, although it can sensitize erythrocytes. Rare strains, such as *E. coli* O:14, produce an immunogenic variant (**Bouazza et al., 2016; Konaré, 2017**).

2. O Antigen (Somatic Antigen)

The O antigen is located in the bacterial outer membrane, specifically in the lipopolysaccharide (LPS) layer, which includes a heat-stable endotoxin. This antigen is resistant to alcohol and acid treatment. When exposed to immune sera containing antibodies against the O antigen, a granular and slow agglutination occurs, which is difficult to disrupt by agitation. In isotonic saline, R-type auto-agglutinating strains may lose their O specificity (**Fauchère & Avril, 2002**).

3. H Antigen (Flagellar Antigen)

Some Enterobacteriaceae express flagella bearing the H antigen. It is encoded by phase-variable genes and used for serotyping within the family. The H antigens are critical for bacterial motility and play a role in tissue colonization and virulence (**Kim & Gadd, 2008; Wilson, 2010**). The H antigen is present only in motile strains. It is composed of the protein flagellin, is heat-stable, and inactivated by alcohol. In the presence of specific antibodies, H antigen elicits a loose, fluffy agglutination that is easily dissociated by agitation (**Bidet & Bingen, 2007**).

4. K Antigen (Capsular Antigen)

The K antigen is a capsular polysaccharide expressed on the surface of certain Enterobacteriaceae. It is frequently associated with bacterial virulence and antibiotic resistance. K antigens help protect bacteria from host immune responses and can mask the O antigen. They are present in *Escherichia coli*, *Shigella*, and some *Salmonella* and *Citrobacter* species (e.g., the Vi antigen, which enhances the virulence of *Salmonella Typhi*) (**Wessels et al., 2018).** These antigens are heat-labile and soluble, and they are destroyed by boiling for 2 hours. Adhesion-related antigens or adhesins—proteinaceous in nature—are also classified under K antigens when associated with pili structures, such as K88 and K99 (**Ferron, 1993**).

I.3. Enterobacteriaceae in Veterinary Medicine

Enterobacteriaceae represent one of the primary causes of infections in both animals and humans and are frequently associated with significant mortality, particularly when resistant strains are involved (**Nordmann, 2011**).

I.3.1. Categories of Pathogens

1. Opportunistic Pathogens

These bacteria are normally commensal inhabitants of the gastrointestinal tract but may become pathogenic under certain conditions such as dysbiosis, physiological stress, or following antimicrobial therapy. The emergence of plasmids carrying resistance genes especially against β-lactams and colistin—has significantly increased the incidence of such infections (**Al-Tawfiq et al., 2017; Aniashi & Dike, 2023**). In dogs, factors such as raw food diets, hospitalization, or recent antibiotic treatment are strongly associated with colonization

by resistant *Enterobacterales* strains (e.g., ESBL or carbapenemase producers) (**Aniashi & Dike, 2023**).

2. Specific Pathogens

Certain species are true animal pathogens, including *Escherichia coli*, *Salmonella*, *Shigella*, *Proteus*, *Klebsiella*, *Enterobacter*, and *Yersinia*, and are implicated in digestive, urinary, or systemic infections depending on the host.

I.3.2. Major Genera and Associated Pathologies

1. Escherichia coli

- **Enteric pathovars** (ETEC, EPEC, STEC, APEC): These strains cause diarrhea in young mammals (e.g., calves, piglets), poultry, and dogs, with clinical presentations ranging from watery diarrhea to septicemia.
- **Extraintestinal pathovars (ExPEC)**: These include UPEC (uropathogenic *E. coli*) responsible for urinary tract infections in dogs, and APEC (Avian Pathogenic *E. coli*), a major agent in avian colibacillosis. Virulence is often enhanced by capsular slime production, particularly in co-infections (**Wikipedia contributors, 2023**).
- **Antimicrobial resistance**: The rise of multidrug-resistant strains is well-documented. ESBL producers are frequently isolated from cattle, pigs, poultry, and companion animals. Resistance to colistin and the presence of carbapenemase genes have also been reported (**Chakraborty et al., 2024; Liu et al., 2016**).

2. *Salmonella* spp.

- Primarily a foodborne pathogen, *Salmonella* is responsible for colibacillosis and septicemia in cattle, pigs, poultry, and horses. The detection of ESBL and carbapenemase- producing strains in animals presents a major public health concern due to their zoonotic potential.

3. *Klebsiella* spp.

- *Klebsiella pneumoniae* causes pneumonia, urinary tract infections, and septicemia, particularly in neonates, elderly animals, or immunocompromised individuals. Multidrug-resistant strains (e.g., resistant to colistin, ESBL, or carbapenems) are increasingly encountered in veterinary medicine (sciencedirect.com; nature.com; bmcinfectdis.biomedcentral.com).(**Paczosa & Mecsas, 2016; Wyres & Holt, 2018; Zhang et al., 2022).**

4. *Proteus*, Morganella, *Providencia* (Family: Morganellaceae)

- These genera are commonly isolated from urinary tract infections, otitis, and septicemia in animals. They are notable for their intrinsic and acquired resistance—particularly to aminoglycosides, fluoroquinolones, and colistin (**O'Hara et al., 2000; Wikipedia contributors, 2023; Zhang et al., 2021**).

5. Enterobacter cloacae

- Frequently implicated in nosocomial infections (e.g., surgical wounds, otitis) among hospitalized animals. There is an increasing prevalence of ESBL and carbapenemase-producing strains (**Davin-Regli & Pagès, 2015; Mezzatesta et al., 2012; Wikipedia contributors,2023).**

Chapter II: Antimicrobial Resistance in Enterobacteriaceae

II.1 Antibiotic Resistance in Enterobacteriaceae

The resistance of Enterobacteriaceae to antibiotics is a longstanding phenomenon that has now emerged as a major global public health concern. Resistance was observed as early as the first decades following the introduction of antibiotics, reflecting the remarkable adaptive capacity of bacteria. This dynamic has been significantly exacerbated by the excessive, inappropriate, or poorly regulated use of antimicrobial agents in both human and veterinary medicine.

This phenomenon extends beyond the issue of therapeutic inefficacy. It represents a cross-cutting threat that affects human health, animal health, and ecological balance—directly aligning with the "One Health" approach.

Among the most affected pathogens are members of the Enterobacteriaceae family, including *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella* spp., and *Enterobacter* spp., which are frequently implicated in multidrug-resistant nosocomial and community-acquired infections.

According to the World Organisation for Animal Health (**WOAH, 2023**), Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) and carbapenemases constitute a critical priority due to their broad resistance spectrum and their capacity to disseminate resistance genes via plasmids. This concern is further emphasized by data from the FAO (2022), which highlights the increased selection pressure in intensive farming systems, where prophylactic and metaphylactic use of antibiotics remains widespread.

According to the World Health Organization (WHO), antimicrobial resistance is defined as the ability of a microorganism to withstand the action of an antibiotic that was previously

effective against it. This phenomenon arises from the capacity of bacteria to tolerate the effects of antimicrobial agents. Depending on the field of study, antimicrobial resistance may be described differently (**Berthuin & Miras, 2022**).

From a microbiological standpoint, a strain is considered resistant when it can grow under conditions where the antibiotic concentration is higher than that tolerated by genetically related susceptible strains (**Lezzar, 2017**). In the pharmacological context, resistance is defined as the inability to achieve an antibiotic concentration at the site of action that is equal to or greater than the minimum inhibitory concentration (MIC) of the bacterium.

In vivo (clinically), a bacterium is classified as resistant when the administered antibiotic treatment fails to cure the infection. This resistance capacity is influenced by several factors, including the anatomical site of the infection, the dosage and route of antibiotic administration, and the immune status of the host (**Muylaert & Mainil, 2012**).

These definitions must also be supplemented by a **genetic definition**: antimicrobial resistance results from a modification in the microorganism's genetic code, leading to the alteration of a gene involved in susceptibility (**Azmoun, 2016**).

II.2. Multidrug Resistance (MDR)

In the literal sense, a multidrug-resistant bacterium (MDRB) is one that exhibits resistance to multiple antibiotics. These bacteria are commonly classified into three categories:

- ✓ **MDR (Multidrug-Resistant):** Bacteria resistant to at least one agent in three or more antimicrobial classes.
- ✓ **XDR (Extensively Drug-Resistant):** Bacteria resistant to all but one or two classes of antibiotics.
- ✓ **PDR (Pan Drug-Resistant):** Bacteria resistant to all available antibiotics.

Bacteria exhibiting multidrug resistance have been defined in various ways. A bacterium is typically classified as multidrug-resistant when it demonstrates resistance to three or more distinct antibiotic classes, one or more classes of critically important antimicrobials, or to a specific key antibiotic.

Methicillin-resistant *Staphylococcus aureus* (MRSA), **multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA)**, and **Extended-Spectrum Beta-Lactamase-producing**

Enterobacteriaceae (ESBL-E) are among the most clinically relevant multidrug-resistant bacteria (**Coustés, 2016**).

In recent years, new multidrug-resistant bacteria (MDRB) have emerged and become increasingly widespread. Unlike the so-called "classical" MDR organisms, these newer strains have acquired significantly higher levels of resistance. They have been classified as **highly drug-resistant emerging bacteria (BHRe)**.

Two main types of bacteria have been identified as BHRe:

- **Glycopeptide-Resistant Enterococci (GRE or VRE – Vancomycin-Resistant Enterococci):** These strains acquire the **Van gene**, which leads to a modification of the antibiotic target by altering the terminal dipeptide of the bacterial cell wall precursor from **D-alanyl-D-alanine** to **D-alanyl-D-lactate**. This change reduces the binding affinity of glycopeptides like vancomycin, rendering them ineffective.

- **Carbapenemase-Producing Enterobacteriaceae (CPE):** These bacteria synthesize **carbapenemase enzymes**, which hydrolyze carbapenems—a class of β-lactam antibiotics with a very broad spectrum of activity, often considered the last line of defense in severe or multidrug-resistant infections.

II.3. Classification of Antimicrobial Resistance

From a genetic standpoint, antimicrobial resistance is divided into two main categories: **intrinsic (ornatural) resistance** and **acquired resistance** (Figure 01).

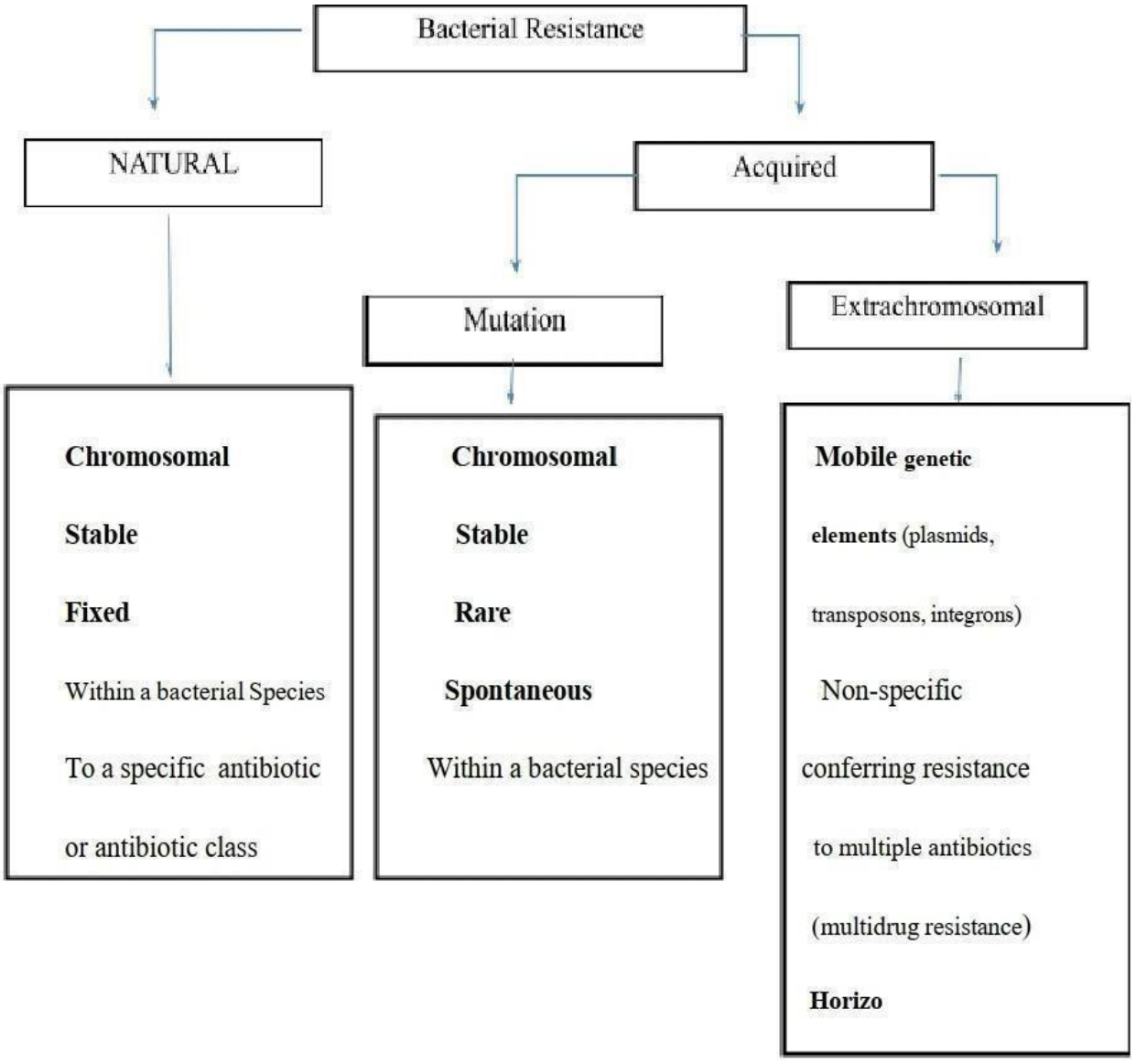

Figure 01 : The two types of antibiotic resistance and their characteristics **(Azmoun, 2016).**

II.3.1. Natural or Innate Resistance

Intrinsic resistance is defined when all strains of a given bacterial species are insensitive to the effect of a specific antibiotic. The persistence of this resistance is inherent to the bacterial chromosome; it is permanent, invariable, and transmitted exclusively by hereditary means.

It therefore represents a constant identification marker for a species, allowing, on one hand, the evaluation of bacterial susceptibility, and on the other hand, the characterization of the wild-type phenotype of a species **(Ivain, 2017)**. This may be due to **(Courvalin, 2008):**

- The absence of target structures, such as the cell wall in *Mycoplasma*, renders *Mycoplasma* species insensitive to antibiotics that act on bacterial cell wall synthesis.
    - The membranes of certain bacteria are impermeable to various antibiotics. For example, the resistance of Gram-negative bacteria to glycopeptides, macrolides, lincosamides, and streptogramins is due to the inabilityof these antibiotics to penetrate the outer membrane.

Enzymatic inactivation of antibiotics, for example through the production of AmpC β- lactamase in certain Enterobacteriaceae.

The following table presents examples of natural resistance in specific bacterial species:

Table 02: Intrinsic Resistance in Various Bacterial Species **(Reygaert, 2018).**

Organisms	**Natural resistance**
Bacteroides spp. (anaerobes)	Aminoglycosides, numerous β-lactams, quinolones
All Gram-positive bacteria	Aztréonam
Enterococci	Aminoglycosides, céphalosporines, lincosamides
Acinetobacter spp.	Ampicilline, glycopeptides
Listeria monocytogenes	Céphalosporines
All Gram-negative bacteria	Glycopeptides, lipopeptides
Escherichia coli	Macrolides
Klebsiella spp	Ampicilline
Serratia marcescens	Macrolides
Pseudomonas aeruginosa	Sulfamides, ampicilline, 1st- and 2nd-generation cephalosporins, chloramphénicol, tétracycline.
Stenotrophomonasmaltophilia	Aminoglycosides, β-lactames, carbapénèmes, quinolones

Enterobacteriaceae can be divided into four groups based on their intrinsic resistance to β-lactam antibiotics:

- ✓ **Group 1 Enterobacteriaceae**: No intrinsic resistance to β-lactams (e.g., *Proteus mirabilis, Escherichia coli, Salmonella, Shigella*).

- ✓ **Group 2 Enterobacteriaceae**: Resistance due to chromosomal penicillinase production (e.g., *Klebsiella spp., Citrobacter koseri*).
- ✓ **Group 3 Enterobacteriaceae**: Resistance mediated by chromosomal cephalosporinase (e.g., *Citrobacter freundii, Enterobacter spp., Serratia spp., Morganella spp., Providencia spp., Hafnia spp.*).
- ✓ **Group 4 Enterobacteriaceae**: Resistance through combined action of a penicillinase and a cephalosporinase (**Mainardi, 2014**).

II.3.2. Acquired Resistance

Acquired resistance affects only certain strains within a given bacterial species. This resistance is no longer considered an inherent trait of the species but rather a characteristic specific to individual strains.

One or more bacterial strains that are naturally susceptible to an antibiotic may suddenly develop resistance due to modifications in their genome (both chromosomal and extrachromosomal). This process is the key mechanism underlying acquired resistance (**Hajjej & Kamoun, 2016; Muller, 2017**).

II.3.2.1. Chromosomal Resistance

The acquisition of chromosomally mediated resistance mechanisms results from a genetic alteration affecting either a structural or regulatory gene.

This process is rare (10 to 20%), occurs spontaneously, and remains stable over time, which explains its specificity to a particular antibiotic or antibiotic class. It is thus characterized by its rarity, specificity, independence, and limited transmissibility (**Mehdi, 2008**).

II.3.2.2. Extrachromosomal Resistance

In contrast, the mechanism of extrachromosomal resistance acquisition is more frequent and more concerning, as it may involve multiple antibiotics , even multiple antibiotic classes simultaneously.

This trait is transmitted via mobile genetic elements (MGEs) such as plasmids, transposons, and integrons, which have the ability to be transferred between bacteria of the same or different species through horizontal gene transfer (HGT) mechanisms: transformation, transduction, and conjugation (**Maurin, 2018; National Order of Pharmacists, 2022**).

1. Conjugation

Conjugation is a mechanism through which genetic information is transferred via the formation of proteinaceous structures (pili, adhesins) following the establishment of physical contact between donor and recipient bacteria.

In Gram-negative bacteria, the increase in surface area of the donor cell, which acts as a bridge toward the recipient cell, facilitates direct contact between the two bacteria. During conjugation, the plasmid replicates, and a single-stranded copy is transferred to the recipient bacterium, where the complementary strand is synthesized. Ultimately, the recipient cell (F−) becomes an F+ cell.

In Gram-positive bacteria, the mechanism differs, particularly in certain bacterial genera such as *Staphylococcus*, where contact with some *Enterococcus* species does not occur via sex pili.

In the external environment, the recipient cell releases pheromones—diffusible peptides—that induce the formation of large aggregates between donor strains (bearing the conjugative plasmid) F+ and F−. This latter step constitutes a gene transfer site, the underlying mechanism of which remains partially unclear (**Khouaja, 2019**).

2. Transformation

Transformation is a mechanism by which certain bacterial species capture extracellular DNA. These bacteria can exhibit specific physiological conditions during their cell cycle—referred to as a state of *competence*—which is required for the binding and uptake of DNA.

Transformation necessitates the presence of free DNA originating from lysed bacterial cells in the environment. The released DNA is bound and absorbed by competent recipient bacteria. In bacterial species such as *Neisseria gonorrhoeae* and *Haemophilus influenzae*, the efficiency of transformation is enhanced by the presence of DNA uptake sequences that are also found within their own genome (**Dutta et al., 2002**).

3. Transduction

Transduction is the transfer of bacterial DNA through bacteriophages (or phages), which are viruses that infect bacteria. These phages can exist in either virulent or temperate forms. Virulent phages multiply within bacterial cells (or are replicated by them) and cause lysis of the host. Temperate phages, on the other hand, integrate into the bacterial chromosome without initiating replication and are replicated synchronously with the bacterial DNA. These integrated bacteriophages are referred to as *prophages*, and the bacteria harboring them are known as *lysogenic bacteria*.

In lysogenic bacterial populations, prophages can occasionally excise themselves from the bacterial chromosome, become highly virulent, replicate, and cause bacterial lysis, thereby infecting new bacterial cells. The transfer of bacterial genes via bacteriophages from one bacterium (lysogeny) to another (lysogeny) can occur if the prophage carries several bacterial genes during its excision (**Khouaja, 2019**).

II.4. Biochemical Mechanisms of Antimicrobial Resistance

Antimicrobial resistance can result from several mechanisms. Biochemically, four main modes of action are described below.

These mechanisms vary depending on the specific bacterial strains (**Chaalal, 2013**):

- An "offensive" strategy through enzymatic inactivation of the antibiotic.
- An "evasion" strategy involving modification of the antibiotic‘s target site.
- A "bypass" strategy through shunting of classical metabolic pathways.
- An "expulsion" strategy involving decreased permeability to the antibiotic and enhanced efflux mechanisms (**Veyssière, 2019**).

These mechanisms may occur individually or in combination, and in the latter scenario, they become significantly more challenging to overcome.

II.4.1. Enzymatic Inactivation of the Antibiotic

Bacteria can produce enzymes that inactivate antibiotics either by chemically modifying them or through hydrolysis. This enzymatic production may be constitutive or induced by external stimuli.

Beta-lactamase is a prime example of such an enzyme produced by bacteria, functioning to inactivate beta-lactam antibiotics by breaking the amide bond within the beta-lactam ring. This represents a highly effective resistance mechanism, transmissible via chromosomal or plasmid-mediated pathways.

New enzymes continue to be discovered. From the 1990s onward, third-generation cephalosporins (3GCs) were introduced into clinical use, but their overuse led to mutations in the genes encoding original β-lactamases. Subsequent mutations emerged rapidly, enabling a range of bacteria to resist higher concentrations of β-lactamase or cephalosporinase, mediated by the **ampC** gene.

These enzymes obstruct the antibiotic's access to its target site. They are typically produced by species that naturally express cephalosporinases, such as certain Enterobacteriaceae and *Pseudomonas* spp.

Following these mutations, the enzyme began to be produced in significantly higher quantities. Some bacteria also produce carbapenemases, which confer resistance to all β-lactam antibiotics, including carbapenems (**Michel-Briand, 2009**).

Among the Enterobacteriaceae, this major resistance mechanism impacts several classes of antibiotics, primarily β-lactams and aminoglycosides, but also quinolones and chloramphenicol (**Pantel, 2016**).

Resistance to Beta-lactams

Enterobacteria can resist β-lactam antibiotics by producing β-lactamases, which hydrolyze the β-lactam ring common to all β-lactams, resulting in inactive compounds (e.g., penicilloic acid derived from penicillin hydrolysis). Consequently, bacteria harboring such enzymes are resistant to one or more β-lactam antibiotics.

There are different types of β-lactamases, distinguished by their affinity for the β-lactam ring. The following are some examples:

- **Penicillinase**: Targets penicillins and, in some cases, first-generation cephalosporins (1GC).
- **Cephalosporinase**: Active against cephalosporins and penicillins.

- **Extended-Spectrum Beta-Lactamase (ESBL)**: A penicillinase that also acts on cephalosporins, functioning synergistically in a way that distinguishes it from cephalosporinases.
- **Carbapenemase**: Also active against carbapenems (**Albano and Moreda, 2016**).

Resistance to Aminoglycosides

Aminoglycosides are bactericidal antibiotics that inhibit protein synthesis at all stages by targeting the 16S rRNA of bacterial ribosomes. The synthesis of modifying enzymes, generally supported by mobile genetic elements (plasmids, transposons, or integron cassettes), constitutes the main mechanism of resistance to aminoglycosides in Enterobacteria. These enzymes are classified according to the reactions they catalyze:

- **Acetylation of an amino group**, referred to as *Aminoglycoside N-Acetyltransferase* (AAC).
- **Phosphorylation of a hydroxyl group**, known as *Aminoglycoside O-Phosphotransferase* (APH).
- **Nucleotidylation of a hydroxyl group**, known as *Aminoglycoside O-Nucleotidyltransferase* (ANT) (**Pantel, 2016**).

II.4.2. Modification of the Antibiotic Target

The targets of antibiotics may be structurally altered or replaced so that antimicrobial compounds can no longer bind to the bacteria and exert their effects. Target modification, a resistance mechanism described for nearly all classes of antibiotics, is particularly significant in resistance to penicillins, glycopeptides, and MLS group molecules in Gram-positive bacteria, as well as to quinolones in both Gram-positive and Gram-negative bacteria.

This type of resistance may result from the acquisition of mobile genetic material encoding enzymes that modify the antibiotic‘s target, or from mutations within the nucleotide sequence of the target itself. Additionally, target replacement is a mechanism observed for β-lactams—particularly sulfonamides and diaminopyrimidines (trimethoprim)—as seen in methicillin-resistant ***Staphylococcus aureus*** (MRSA), and across all veterinary β-lactams.

A notable example is the synthesis of a novel penicillin-binding protein (PBP) with low affinity for methicillin.

Target modifications can occur in two main forms:

- **Quantitative modifications**: For example, the absence of a cell wall in *Mycoplasma* species confers natural resistance to β-lactam antibiotics (**Pascale, 2014**).
- **Qualitative modifications**: Alteration in the structure of a target protein can reduce its affinity for antibiotics. This represents a common mechanism of acquired resistance (**Pascale, 2014**).

Resistance to β-lactams

This is due to a modification of the "penicillin-binding protein" (PBP). PBPs are enzymes that catalyze the final step in the biosynthesis of peptidoglycan (bacterial cell wall) and represent a primary target for β-lactam antibiotics. By binding to PBPs, β-lactams prevent these enzymes from performing their function, thereby inhibiting peptidoglycan synthesis. Three main mechanisms may be involved:

- **Reduced affinity of PBPs for β-lactams**: β-lactams have difficulty binding to PBPs, which remain available to catalyze peptidoglycan synthesis.
- **Increased synthesis of native PBPs**: Overexpression of PBPs that naturally exhibit low affinity for β-lactams.
- **Synthesis of one or more novel PBPs**: These newly synthesized PBPs are insensitive to β-lactams (**Lozniewski and Rabaud, 2010**).

Resistance to Quinolones

Acquired resistance to quinolones is largely attributed to chromosomal mutations within the genes encoding their intracellular targets, specifically type II topoisomerases—DNA gyrase (composed of GyrA and GyrB subunits) and DNA topoisomerase IV (ParC and ParE). In Enterobacteriaceae, mutations most commonly affect the **gyrA** gene, followed by **parC**, targeting a highly conserved region of both genes known as the **Quinolone Resistance Determining Region** (QRDR). These genetic alterations result in changes to the secondary and tertiary structures of the GyrA and ParC subunits, reducing the binding affinity of the DNA-enzyme complex for quinolones. Resistance levels are progressive and correlate with the number of mutations detected in the QRDR (**Pantel, 2016**).

II.4.3. Decreased Antibiotic Concentration

In this mechanism, the antibiotic itself remains unaltered, but an insufficient amount reaches its target site (**Veyssiere, 2019**). A commonly utilized bacterial strategy to mitigate antibiotic efficacy involves the **induction or overexpression of drug efflux pumps**. As their name implies, these integral membrane proteins actively export antimicrobial agents from the bacterial cytoplasm into the extracellular environment, thereby maintaining sub-therapeutic intracellular concentrations of the antimicrobial compounds (**Giedraitiene et al., 2011; Richardson, 2017; Varela et al., 2021**).

Antimicrobial Efflux Transporters Are Currently Classified into Five Families

Some efflux pumps selectively export specific antibiotics, whereas others—known as **MDR (multidrug resistance) pumps**—transport a broad array of structurally diverse compounds with different antimicrobial modes of action (**Lomovskaya and Watkins, 2001**).

Resistance to β-lactams

The **active efflux system** is mediated by transmembrane proteins embedded both in the inner and outer membranes of Gram-negative bacteria. Mutations within the regulatory regions of multidrug efflux operons can lead to the **overexpression of constitutive efflux systems**, often in combination with **porin loss**, conferring broad-spectrum antibiotic resistance. The role of efflux systems in β-lactam resistance has been clearly demonstrated in several studies, particularly in *Klebsiella pneumoniae*.

This mechanism primarily affects **second-generation cephalosporins** (**Walsh, 2003**).

II.4.4. Reduced Cell Envelope Permeability

Most antibiotics must penetrate bacterial cells to reach their intracellular targets. **Porin channels** are the main entry routes through the outer membrane of bacteria. Resistant bacteria reduce the permeability of either the inner or outer membrane to **impede antibiotic uptake**. This mechanism is especially relevant in **Gram-negative bacteria** and restricts the entry of toxic compounds from the external environment, such as β-lactams, tetracyclines, and certain fluoroquinolones (**Srijana, 2021**).

Resistance to β-lactams

Penetration of β-lactams—hydrophilic molecules—across the outer membrane occurs via **porins**, which are aqueous protein channels. Thus, susceptibility to β-lactams is directly dependent on the **quantity of functional porins**.

Mutational changes in porins lead to acquired resistance to β-lactams either through s**ignificant structural alterations** of the porin, as observed in *Escherichia coli*, or **quantitative reduction** in porin expression, which is the most frequently encountered scenario (**Kumar and Schweizer, 2005**).

II.4.5. Other Mechanism: "Altruism"

To protect susceptible bacteria and promote the elimination of antibiotics, highly resistant bacteria can synthesize large quantities of **indole**. This organic compound exerts a dual role in resistance: it facilitates the **elimination of antibiotics** and activates **metabolic pathways that inhibit the production of reactive oxygen species (ROS)**, which are otherwise induced by antibiotics (**Veyssiere, 2019**).

Chapter III: The Dromedary and Its Role in the "One Health" Concept in the Context of Antimicrobial Resistance

The ―One Health‖ concept is based on the interconnection between human health, animal health, and environmental health. This integrated approach is particularly relevant in addressing emerging threats such as antimicrobial resistance. In this context, domestic animals including less conventional species such as the dromedary (*Camelus dromedarius)* may play a significant role in the circulation, persistence, and dissemination of antibioticresistant bacteria.

III.1. The Dromedary: A Key Animal in Agro-Pastoral Systems

Present in the arid and semi-arid regions of North Africa, the Middle East, and Asia, the dromedary (*Camelus dromedarius*) is a valuable species from an economic, nutritional, and health standpoint.

In Algeria, particularly in the Saharan and pre-Saharan regions, it plays a vital role in food security and the livelihoods of pastoral communities. As a source of milk, meat, leather, and transportation, the dromedary actively contributes to the resilience of rural populations living under extreme climatic conditions. However, its potential role in the dynamics of pathogenic agents , especially antibiotic-resistant bacteria remains largely underestimated.

Recent research conducted in Algeria and other North African countries has revealed that dromedaries can harbor antibiotic-resistant Enterobacteriaceae, notably *Escherichia coli*,

Salmonella spp., *Klebsiella pneumoniae*, along with other multidrug-resistant strains. Some of these bacteria produce extended-spectrum β-lactamases (ESBL), complicating their treatment (**Younan et al., 2021; Harir et al., 2022**).

In Algeria, resistant isolates have been identified from camel milk, meat, or feces, confirming the presence of resistance genes against antibiotics that are critical to human health, such as third-generation cephalosporins or colistin. These findings underscore the importance of integrating the dromedary into antimicrobial resistance surveillance systems within the framework of the ―One Health‖ concept especially in Algeria, where this species is strategically linked to public health, food security, and the management of Saharan ecosystems.

III.2. The Dromedary as a Potential Reservoir of Antimicrobial-Resistant Bacteria

The dromedary is often raised in environments where sanitary conditions and antibiotic stewardship are poorly regulated. Practices such as self-medication, empirical veterinary use **Cultural characteristics of Enterobacteriaceae on selective media** of antimicrobials without prior antibiogram testing, and transboundary livestock trade increase the risk of exposure to multidrug-resistant (MDR) strains. Furthermore, pastoral practices including herd aggregation and shared access to water sources facilitate horizontal gene transfer (HGT) of resistance determinants, particularly via plasmids (**El-Diasty et al., 2023**).

Recent studies have isolated antimicrobial-resistant Enterobacteriaceae in dromedaries, including resistance to critically important antibiotics for human medicine such as colistin, fluoroquinolones, and carbapenems representing a significant public health concern (**Kebe et al., 2023**). These bacteria can be transmitted to humans through direct contact, consumption of raw milk or undercooked meat, or via environmental contamination.

III.3. Integration of the Dromedary within the "One Health" Framework

The ―One Health‖ approach advocates for integrated surveillance of antimicrobial resistance across humans, animals, and the environment. Within this context, the dromedary being a semi-nomadic species frequently interacting with both anthropogenic and natural ecosystems must be included in epidemiological surveillance networks.

Experimental Section

Experimental Section

Objectives

In a context where the fight against bacterial infections is becoming increasingly complex, the emergence of **antimicrobial resistance (AMR)** represents a major challenge for both human and animal public health. In this regard, our study contributes to the monitoring and understanding of this phenomenon in the Saharan environment.

The specific objectives of this study are as follows:
▪ To isolate and identify **Enterobacteriaceae** strains present in the fecal matter of dromedaries sampled from various regions of the Algerian Sahara;

▪ To assess the **antibiotic resistance profiles** of the isolated bacterial strains, with the aim of detecting the potential presence of **multidrug-resistant** isolates;

▪ To characterize the **intestinal Enterobacteriaceae microbiota** of dromedaries in connection with local livestock management practices, with a view to preventing and managing **antimicrobial risk**.

I. Materials and Methods

I.1. Study Site

Microbiological analyses were conducted at the **Clinical Microbiology Laboratory** of the **École Nationale Supérieure Vétérinaire (ENSV)** in Algiers. This laboratory is equipped for bacterial culture, biochemical identification, and **antimicrobial susceptibility testing (AST)**.

I.2. Duration and Type of Study

This experimental study was carried out over a period of two months, from April to June 2025. It focused on the isolation, identification, and antimicrobial susceptibility evaluation of **Enterobacteriaceae** strains recovered from dromedary fecal samples.

I.3. Sampling

A total of 50 samples of fresh fecal matter were aseptically collected from dromedaries in the Ouargla region, located in southeastern Algeria

Figure 02: Fresh fecal matter collected from dromedaries **(personal photograph).**

Samples were obtained using sterile gloves, either directly from the rectum or immediately after defecation, and then transferred into sterile containers.

The samples were transported in an **isothermal cooler** maintained at +4 °C and delivered to the laboratory within a maximum time frame of 24 hours.

I.1. Non-Biological Materials

I.1.1. Equipment and Small Laboratory Supplies

- Light microscope
- Incubator set at 37 °C
- Sample collection tubes (plastic tubes with labeling area available)
- Tube rack
- Antibiotic susceptibility testing (AST) disks
- Caliper
- Bunsen burner
- Inoculating loop (platinum wire loop)
- Petri dishes
- Microscope slides and cover slips
- Vortex mixer
- Autoclave
- Sterile distilled water
- Immersion oil
- Lugol‘s iodine solution
- Crystal violet
- Alcohol
- Basic fuchsin

I.1.2. Culture Media

- Hektoen enteric agar
- Mueller–Hinton agar
- MacConkey agar
- Triple Sugar Iron (TSI) agar
- Mannitol motility medium
- Urea-Indole liquid medium
- Sterile physiological saline solution
- Nutrient broth (Brain Heart Infusion Broth [BHIB], Peptone water)
- API 10S biochemical identification gallery

I.1. Methods

I.1.1. Enrichment

The fecal samples were cultured in **Brain Heart Infusion Broth (BHIB)** and incubated at 37 °C for 24 hours. This enrichment step allows the activation and proliferation of bacteria present in the samples prior to inoculation on solid media.

I.1.2. Isolation of Bacterial Strains

From the enriched BHIB cultures, bacterial isolation was performed on various **selective media specific for Enterobacteriaceae** in order to obtain pure cultures.

- Each colony type was subcultured on the same selective medium using streak plating with a **platinum inoculating loop** or a **Pasteur pipette**, creating well-spaced streaks.
- Plates were incubated at 37 °C for 24 to 48 hours.
- After incubation, it was essential to verify that the colonies retained the same **macroscopic and microscopic characteristics** as those initially observed.
- Subculturing was repeated as needed until a **pure isolate** displaying consistent characteristics with the original isolate was obtained.

Each pure culture underwent the following analyses:

- **Macroscopic examination**
- **Gram staining**
- **Comprehensive biochemical identification**
- **Antibiotic susceptibility testing (antibiogram)**

I.1.3. Macroscopic Examination

The **macroscopic evaluation of Enterobacteriaceae** consisted of visual inspection of bacterial colonies on selective culture media.

This involved assessing characteristics such as colony size, shape, odor, color, margin definition, and texture, as well as any **color change or indicator reaction in the selective media**, which aids in presumptive identification of the bacterial species.

Table 03: Cultural Characteristics of Enterobacteriaceae on Selective Media

Selective Medium	Microorganisms	Colony Morphology
Mac Conkey	-*Escherichia coli* (E. coli) -*Salmonella* spp., *Shigella* spp., and others -*Enterobacter* spp., *Klebsiella* spp.	-Large, red colonies -Colorless, transparent colonies -Large, pink, mucoid colonies
Hektoen	-*Escherichia coli* (E. coli), *Citrobacter* spp., *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Arizona* spp. -*Citrobacter freundii, Proteus vulgaris* -*Proteus mirabilis, Salmonella* spp. -*Shigella* spp., *Providencia* spp., *Proteus morganii, Proteus rettgeri, Salmonella* spp. (non-H_2S-producing)	-Salmon-pink colonies -Salmon-pink colonies with black centers -Blue or green colonies with black centers -Whitish or green colonies
SS	-*Salmonella* spp. (H_2S-positive), *Proteus vulgaris, Proteus mirabilis* -*Salmonella* spp. (H_2S-negative), *Shigella* spp., *Serratia* spp., *Proteus morganii* -*Proteus rettgeri, Providencia* spp.	-Colorless colonies with black centers -Colorless, transparent colonies -Colonies with orange centers

I.5.4 Microscopic Examination

Microbiological identification methods may be selected based on the results of microscopic analysis.

This examination is conducted either via **wet mount preparation**, a rapid method involving the observation of a bacterial suspension between a slide and coverslip under a ×40 objective lens. This observation primarily provides information regarding bacterial motility. The type of flagellation (e.g., monotrichous, peritrichous, etc.) may be inferred based on the motility pattern, if present, which assists in the preliminary orientation of the bacterial isolate.

Alternatively, **bacterial staining** techniques may be employed to visualize bacterial morphology and to classify bacteria into two groups—Gram-negative and/or Gram-positive—based on their ability to retain the crystal violet stain under specific staining conditions (see Appendix 01).

Typically, members of the family *Enterobacteriaceae* are Gram-negative bacilli and thus appear **pink** following Gram staining.

II. Identification Using Classical and Mini Biochemical Test Strips (API 10S)

Classical biochemical galleries are used to perform the biochemical identification of purified bacterial isolates.

The primary identification characteristics of *Enterobacteriaceae* include their **motility**, ability to **ferment carbohydrates**, **utilization of citrate**, and production of specific **metabolites from glucose fermentation**.

II.1. Oxidase Test

This test constitutes a fundamental tool for the identification of Gram-negative bacteria. It is based on the bacterial production of the enzyme *cytochrome oxidase* (more specifically *phenylenediamine oxidase*), which functions within the electron transport chain.

Principle

The test is designed to demonstrate the ability of bacteria to oxidize a colorless reagent (*N,N-dimethyl-p-phenylenediamine*) into a violet-pink compound.

Procedure

- Using flame-sterilized forceps, place a piece of filter paper impregnated with *N,N-dimethyl-p-phenylenediamine* onto a microscope slide.
- Using a Pasteur pipette, collect a bacterial colony grown on solid medium and gently apply it to the prepared paper.

Interpretation

- If the colony turns pink or violet, the organism produces oxidase, and the test is considered **positive**.
- If the colony remains colorless, the organism lacks oxidase activity, and the test is considered **negative**.

II.2. Catalase Test

This test is a basic procedure used for the identification of Gram-positive bacteria.

Principle

Catalase is an enzyme abundantly produced by bacteria with aerobic or facultative anaerobic metabolism that are capable of degrading hydrogen peroxide (H_2O_2), a compound toxic to bacterial cells. This test is based on the enzymatic breakdown of hydrogen peroxide into water and oxygen according to the following reaction:

$$H_2O_2 \longrightarrow 1/2O_2+H_2O$$

Technique

- On a clean, dry glass slide, add a few drops of 10-volume hydrogen peroxide (H_2O_2).
- Using a platinum inoculation loop or a Pasteur pipette, apply the bacterial inoculum to the drop.
- Observe the reaction **immediately** with the naked eye.

Interpretation

- **Gas release (bubbling)**: Indicates the liberation of oxygen (O_2) during the breakdown of hydrogen peroxide (H_2O_2), characterizing the strain as **catalase-positive**.
- **Absence of gas release**: Indicates the lack of oxygen (O_2) production during the breakdown of hydrogen peroxide (H_2O_2), characterizing the strain as **catalase-negative**.

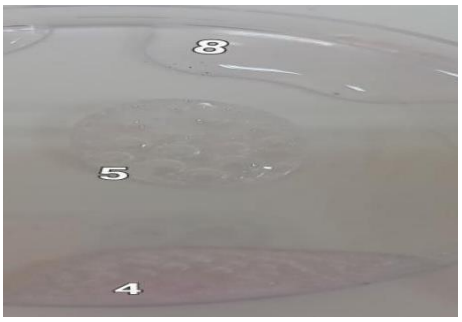

Figure 03 : Catalase Test **(personal photograph).**

II.3. Investigation of Glucose and Lactose Utilization, Gas and H_2S Production on TSI Medium

Principle

Triple Sugar Iron (TSI) agar is used to guide the identification of Enterobacteriaceae. It is a differential medium that allows the detection of glucose, lactose, and/or sucrose fermentation, as well as the production of gas and hydrogen sulfide (H_2S).

Technique

-Using a Pasteur pipette or a platinum inoculating loop, collect a few drops of the bacterial suspension. Inoculate the slanted surface by streaking in close zigzags and stab the butt of the tube vertically.

-Incubate at 37 °C for 24 hours.

-The use of a sugar causes the phenol red indicator to turn yellow.

Interpretation

Carbohydrate (glucose, sucrose, lactose) fermentation results in acid production, detected bya color change of the phenol red indicator from red to yellow:

- Positive lactose fermentation: yellow coloration of the slant.
- Positive sucrose fermentation: yellow coloration of the middle zone.
- Positive glucose fermentation: yellow coloration of the butt of the tube.

The presence of gas is indicated by bubble formation or displacement of the agar medium.

H_2S production is evidenced by the appearance of a black precipitate.

Figure 04: TSI Agar (Triple Sugar Iron Agar)

A : TSI + with H_2S Production **B:** TSI **agar** –

II.4. Mannitol Motility Test

Principle

This test utilizes a semi-solid medium containing mannitol and phenol red as pH indicators. It is designed to assess both the ability of bacteria to ferment mannitol, which leads to acidification of the medium, and their motility.

Technique

-Using a sterile Pasteur pipette or a platinum inoculation loop, inoculate the medium by a single central stab.

Incubate at 37°C for 18–24 hours. This medium is applicable only for fermentative bacteria.

-Results are interpreted by direct visual examination.

Reading

-Positive reaction: Mannitol fermentation is indicated by a color change of the pH indicator from red to yellow. Bacterial motility is evidenced by diffuse turbidity radiating from the stab line.

-Negative reaction: No color change; the medium remains red and clear along the stab line, indicating absence of fermentation and motility.

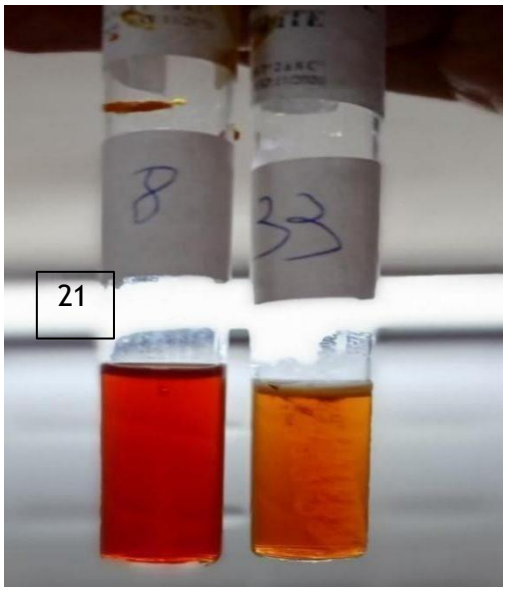

Figure 05: Mannitol Motility Test — 01(-) and 02(+) with motility **(personal photo)**

II.5.Detection of β-galactosidase: ONPG Test

Principle

The ONPG test is used to detect the presence of the enzyme β-galactosidase. Instead of lactose, a different β-galactoside is used as the substrate: ortho-nitrophenyl-β-D-galactopyranoside (ONPG). The test detects the enzyme's ability to break down lactose into glucose and galactose.

Technique

-Prepare a concentrated bacterial suspension in 0.5 ml of sterile water. The bacteria should be taken from a culture grown on MacConkey agar.

-Aseptically place a paper disc impregnated with the ONPG substrate into the suspension.

-Incubate at 37°C, and monitor every 15 minutes for up to one hour.

Reading

-A **positive result** is indicated by the appearance of a stable yellow color, due to the presence of ortho-nitrophenol (ONP), which results from ONPG hydrolysis.

-A **negative result** shows no color change, indicating that ONPG was not hydrolyzed and that β-galactosidase is absent.

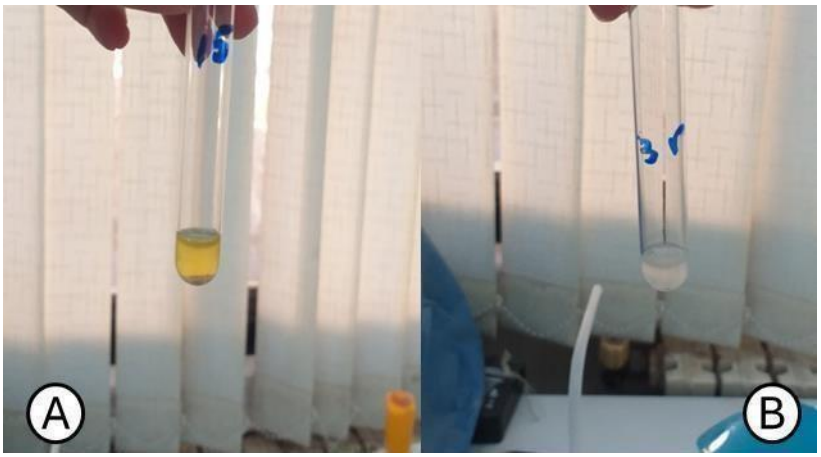

Figure 06: ONPG test (A - negative, B + positive)

II.6. Methyl Red (MR) and Voges-Proskauer (VP) Tests

Principle Clark and Lubs medium can be used to study the fermentation pathways of Enterobacteriaceae and to distinguish between *mixed acid fermentation* and *butylene glycol fermentation*.

-The MR test: Uses methyl red to detect mixed acid fermentation, which strongly acidifies the medium following glucose fermentation.

-The VP test: Detects the production of acetoin (3-hydroxybutanone), a neutral end product of butylene glycol fermentation. In the presence of a strong base (potassium hydroxide or sodium hydroxide) and α-naphthol, acetoin turns red in an oxygen-rich environment.

Technique

-Inoculate Clark and Lubs medium with the test organism.

-Incubate for 24 hours at 30°C.

-After incubation, divide the culture into two tubes to perform both tests:

In the **first tube**, add methyl red indicator.

In the **second tube**, add a few drops of KOH or NaOH (VP1 reagent) and 10 drops of α-naphthol (VP2 reagent). Tilt the tube to enhance oxygenation and observe for up to one hour.

Reading

-MR Test (first tube):

Yellow color (MR–): the pH is only mildly acidic.

Red color (MR+): the pH is strongly acidic, indicating mixed acid fermentation.

-VP Test (second tube):

Red or pink color at the surface (which may diffuse through the medium): indicates the presence of acetoin (VP positive bacteria).

Figure 07: MR Test -

Figure 8: MR Test +

Figure 9: VP Test -

II.7. Detection of Indole and Urease Activity

Principle:

The **urea-tryptophan medium**, often mistakenly called *urea-indole*, is a synthetic medium used in bacteriology that allows for the simultaneous detection of:

- **Indole production** (via hydrolysis of tryptophan by the enzyme tryptophanase)
- **Urease activity** (via hydrolysis of urea)

Technique:

-Heavily inoculate the urea-indole medium with several colonies of the test strain.

-Incubate at 37°C for 24 hours.

-After incubation, add a few drops of **Kovac's reagent** and interpret the results.

Reading:

-Urease production: The medium turns **pink to violet**.

-Indole production: A **red ring** appears at the surface of the medium.

Figure 10: Urea-Indole Test **(personal photo)**

III. API S10 Gallery

The **API 10S gallery** (BioMérieux) is a ready-to-use strip containing **10 dehydrated substrates** that allows for the performance of **10 biochemical tests** to identify **Gram-negative bacilli** belonging to the **Enterobacteriaceae family**.

This is a simplified version of the API 20 E gallery, offering 10 tests instead of 20. (The **oxidase reaction** is considered the **11th test**, and **nitrate reduction to nitrites (NO_2)** is considered the **12th**.)

Inoculation procedure:

First, the base and lid of a specific incubation tray are prepared. Approximately **3 mL of distilled water** is added into the humidity chamber (alveolus) to create a **moist atmosphere**. Then, the API strip is placed in the incubation tray.

Using a **gallery slide** and a **Pasteur pipette**, one or two **well-isolated colonies** of the Gram-negative organism are picked. A **bacterial suspension** is made by thoroughly homogenizing the colonies.

The **same pipette** is then used to **dispense the bacterial suspension** into the microtubes of the gallery. To **avoid air bubbles**, the pipette tip is placed on the **side wall** of each well while slightly **tilting the incubation box forward**.

- For the **citrate utilization test**, both the **tube and cupule** are filled.
- For the other tests, **only the tube** is filled.

Anaerobic conditions are required for the LDC, ODC, UREA, and H_2S tests; this is achieved by **covering the cupules with mineral oil** (paraffin oil).

The tray is then **sealed** and **incubated at 37°C for 24 hours**.

Reading and interpretation:

After incubation, the gallery is interpreted using a **reading table** (see Appendix 03).

Identification is carried out either by:

- A **dichotomous method** using **API numerical profiling**, or
- A **probabilistic method** using **software**.

In this study, **numerical profiling** was used for identification.

Figure 11: Inoculation technique for the API S10 gallery **(personal photo)**

IV. Antibiogram

The purified strains were subjected to **antibiotic susceptibility testing** using a **standard disc diffusion method** on **Mueller-Hinton (MH) agar**, in accordance with **CLSI guidelines (CLSI, 2020)**.

IV.1. Principle

Filter paper discs impregnated with specific antibiotics are placed on the surface of an agar plate that has been inoculated with a **pure culture of the strain** to be tested. The **antibiotics used** in this study are listed in the following table:

Table 04: Antibiotics tested for Enterobacteriaceae

Family		Antibiotics	Dosage (µg)
β-lactams	Penicillin A	Amoxicillin (**AML**)	25 µg
		Amoxicillin + Clavulanic acid (**AMC**)	30 µg
	Carboxypenicillin	Ticarcillin (**TIC**)	75 µg
		Ticarcillin + Clavulanic acid (**TCC**)	75+10 µg
	Ureidopenicillin	Piperacillin (**PRL**)	100 µg
	1st Gen Cephalosporin	Cephalothin (**KF**)	30 µg
	2nd Gen Cephalosporin	Cefoxitin (**FOX**)	30 µg
	3rd Gen Cephalosporin	Cefotaxime(**CTX**) Ceftazidime (**CAZ**)	30 µg

	Carbapenem	Imipenem (**IMP**)	10 µg
	Monobactam	Aztreonam (**AT**)	30 µg
Aminoglycosides		Gentamicin (**CN**)	10 µg
		Tobramycin (**TOB**)	10 µg
		Amikacin (**AK**)	20 µg
Fosfomycine		Fosfomycine **(FF)**	200 µg
Tetracyclines		Tetracycline (**TE**)	30 µg
Fluoroquinolone		Ciprofloxacine (CIP)	5 µg
Polymyxine		Colistine (CT)	10 µg

Immediately after the application of the antibiotic disks, the antibiotics diffuse uniformly, with their concentration decreasing in inverse proportion to the distance from the disk. Bacterial growth ceases where the antibiotic concentration in the agar medium reaches the Minimum Inhibitory Concentration (MIC).

The resistance or susceptibility profile of the strain will thus be determined (**Rahal et al., 2014**).

IV.2. Technique

Medium for Antimicrobial Susceptibility Testing

Mueller-Hinton agar is poured into sterile Petri dishes and must be dried prior to use.

Preparation of the Inoculum

-Following the revival of the studied strains* and from a pure culture developed on a suitable isolation medium for 18 to 24 hours, well-isolated and morphologically identical colonies are collected using a sterile swab.

-The loop or swab is discharged into 5 to 10 mlof sterile physiological saline solution (0.9%).

The bacterial suspension is then homogenized to achieve a turbidity equivalent to 0.5 McFarland units.

The inoculum can be adjusted by adding either more culture if too weak, or sterile physiological saline if too concentrated.

Inoculation must be carried out within 15 minutes of preparing the inoculum.

Inoculation

A sterile and dry swab is immersed into the bacterial suspension and then gently pressed against the internal wall of the tube to remove the excess liquid.

The swab is then streaked over the entire surface of the dried agar in a back-and-forth motion using tight zigzag lines.

The operation is repeated three times with a 60° rotation of the plate each time, ensuring the swab is rotated on itself. Inoculation is concluded by swabbing along the periphery of the agar surface.

The swab must be reloaded each time a Petri dish is inoculated.

Application of Antibiotic Disks

It is recommended not to place more than 6 antibiotic disks on a 90 mm diameter Petri dish. The antibiotic disks are placed in the center, approximately 25 mm from the center to center.

Each antibiotic disk is picked up with a sterile forceps and deposited on the inoculated Mueller-Hinton agar surface.

Each disk should be pressed gently to ensure proper adherence. Once applied, the disk must not be moved.

Incubation and Reading

The Petri dishes are incubated at 37°C for 18 to 24 hours.

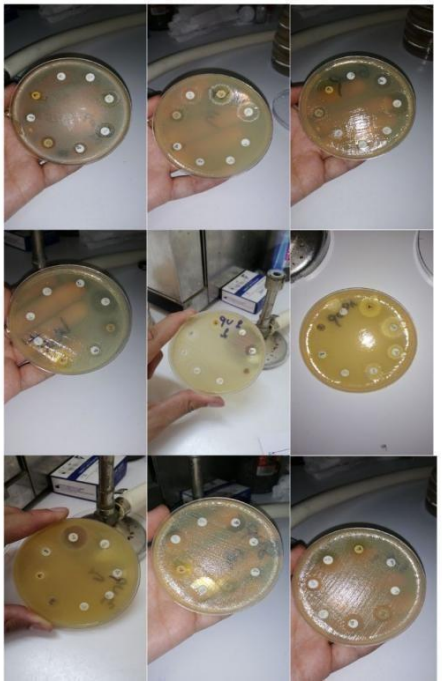

figure 12: Examples of agar media showing inhibition spectra of various antibiotics **(personal photograph).**

The evaluation of antimicrobial resistance in the isolates is based on the precise measurement of the diameters of the inhibition zones using a metal caliper.

Measurements are performed by viewing through the bottom of the closed Petri dish.

These results are compared to the critical values for inhibition zone diameters and Minimum Inhibitory Concentrations (MICs) specified for Enterobacteriaceae, as indicated in the standardized reading tables for antibiogram interpretation from the national scale—Human and Veterinary Medicine, 7th Edition (2014).

The isolates are ultimately classified as: susceptible (S), intermediate (I), or resistant (R) (Annexes 04).

Figure 13: Measurement of inhibition diameter using a caliper **(personal photograph).**

V. Multiple Antibiotic Resistance (MAR) Index Calculation

Among the isolated Enterobacteriaceae, the MAR index is defined as *a/b*, where ―a‖ represents the number of antibiotics to which the isolate was resistant, and ―b‖ represents the total number of antibiotics to which the isolate was exposed.

The method for determining multiple antibiotic resistance (MAR) was analyzed following the guidelines of Krumperman (1983).

The MAR index was calculated by dividing the number of antibiotics to which the tested isolate was resistant by the total number of antibiotics used to evaluate its susceptibility.

VI. Workplace

The isolation, identification, and antibiotic susceptibilitytesting of Enterobacteriaceae were conducted in the Clinical Microbiology Laboratory of the National Higher School of Veterinary Medicine (ENSV – Algiers).

VI.1. Duration and Type of Study

This work was carried out over different periods and comprises:

- A retrospective studyconducted over a period of one year and three months, spanning from May 2022 to July 2022.
- Aprospective study carried out over two months, from April to June 2023.

The objective of this study was to analyze the identification and antibiogram results of bacterial isolates belonging to the Enterobacteriaceae family, isolated from various foodstuffs.

VI.2. Materials

VI.2.1. Biological Material

A total of 46 strains belonging to the Enterobacteriaceae family, obtained from previous studies conducted by Dr. Guessoum and her students in various graduation projects, were isolated and preserved in a conservation medium.

The origin of the various Enterobacteriaceae strains analyzed in our study, coming from different regions of the Wilaya of Algiers, is presented in Table 03 below.

Figure 14: Bacterial strains studied on inclined nutrient agar **(Personal photograph).**

Preservation of Strains

The Enterobacteriaceae strains were preserved in duplicate in broth (Tryptic Soy Broth and glycerol) at -20°C in the freezer and on maintenance agar at +4°C in the refrigerator.

All 46 preserved and analyzed Enterobacteriaceae strains originated from different samples:

- 15 strains collected from the Clinical Microbiology Laboratory are distributed as follows: 5 isolated from broiler chicken samples and 5 from fish samples.
- 14 strains collected from the Pasteur Institute Laboratory, isolated from red minced meat (08) and Merguez sausage (06).

Table 05: Origin of various bacterial strains

Food Source	Number of Strains
Fishery products	15
Broiler chicken (meat)	17
Minced meat	8
Merguez sausage	6
Total	46

VI.2.2 Non-biological Material

1. Equipment and Small Tools

- Optical microscope
- Incubator set at 37 °C
- Tubes (swabs supplied in plastic tubes with labeling sticker, available with wooden, plastic, aluminum, or paper stems, with synthetic or natural sterile tips)
- Tube rack
- Antibiogram discs
- Vernier caliper
- Bunsen burner
- Platinum loop
- Petri dishes
- Slides and cover slips
- Vortex mixer
- Autoclave
- Sterile distilled water
- Immersion oil
- Lugol‘s iodine
- Crystal violet
- Alcohol
- Basic fuchsin

2. Culture Media

- Hektoen agar
- Mueller-Hinton agar
- MacConkey agar
- Triple Sugar Iron (TSI) agar
- Motility Mannitol medium
- Urea-Indole liquid medium
- Sterile physiological saline
- Nutrient broth (BHIB, Peptone water
- API 10S identification gallery

VI.3. Methods

VI.3.1. Re-verification of Preserved Strains

The preserved strains underwent re-verification in nutrient broth (BHIB), as shown in **Figure 15**, followed by incubation at 37 °C for 24 hours.

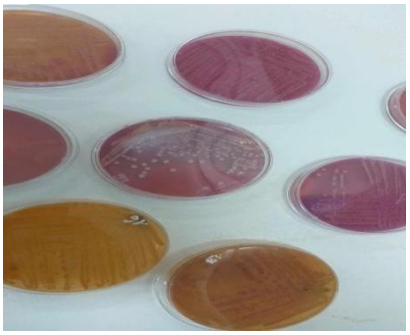

Figure 15: Revivification of the studied bacterial strains **(personal photo)**

VI.3.2. Re-isolation of Strains (Purification)

From the nutrient broth, a re-isolation was performed on various selective media for Enterobacteriaceae to obtain a pure culture.

- Each type of colony must be subcultured on the same isolation medium by creating spaced streaks using a platinum loop or a Pasteur pipette.
- Incubate at 37°C for 24 to 48 hours.
- After incubation, it is essential to verify that the colonies exhibit the same microscopic and macroscopic characteristics as when initially isolated.
- Continue subculturing until a pure isolate exhibiting the same features as those from the initial isolation is obtained.

Each pure culture underwent:

- Macroscopic examination
- Gram staining
- Complete biochemical identification
- Antibiotic susceptibility testing (Antibiogram)

VI.3.3. Macroscopic Examination

The macroscopic examination of Enterobacteriaceae involves visual observation of bacterial colonies on culture media.

It consists of evaluating characteristics such as size, shape, odor, color, edge appearance, and texture of the colonies, as well as the color change in the selective culture media used to identify these bacteria.

Table 06: Cultural characteristics of Enterobacteriaceae on selective media

Medium	Microorganisms	Colonies
MacConkey	*E. coli Salmonella, Shigella,* others *Enterobacter, Klebsiella*	Large, red Colorless, transparent Large, pink, mucoid
Hektoen	*E. coli, Citrobacter, Klebsiella, Enterobacter, Serratia, Arizona Citrobacter freundii, Proteus vulgaris Proteus mirabilis, Salmonella Shigella, Providencia, Proteus morganii, Proteus rettgeri*, H_2S-negative *Salmonella*	Salmon-pink Salmon-pink with black center Blue or green with black center Whitish or green
SS (Salmonella-Shigella)	H_2S-positive *Salmonella, Proteus vulgaris* and *mirabilis* H_2S-negative *Salmonella, Shigella, Serratia, Proteus morganii Proteus rettgeri, Providencia*	Colorless with black center Colorless, transparent Colonies with orange center

VII. Results

VII.1. Results of Isolation and Biochemical Identification of Enterobacteriaceae

Out of the 40 camel fecal samples analyzed, 33 bacterial isolates belonging to the Enterobacteriaceae family were recovered, corresponding to an overall isolation rate of **82.5%**.

The isolates were identified based on their morphology, Gram stain characteristics (Gram-negative bacilli), and biochemical profiles. Identification was confirmed using conventional tests as well as the API 10S identification system (bioMérieux®).

The identified Enterobacteriaceae isolates belonged to the following genera:

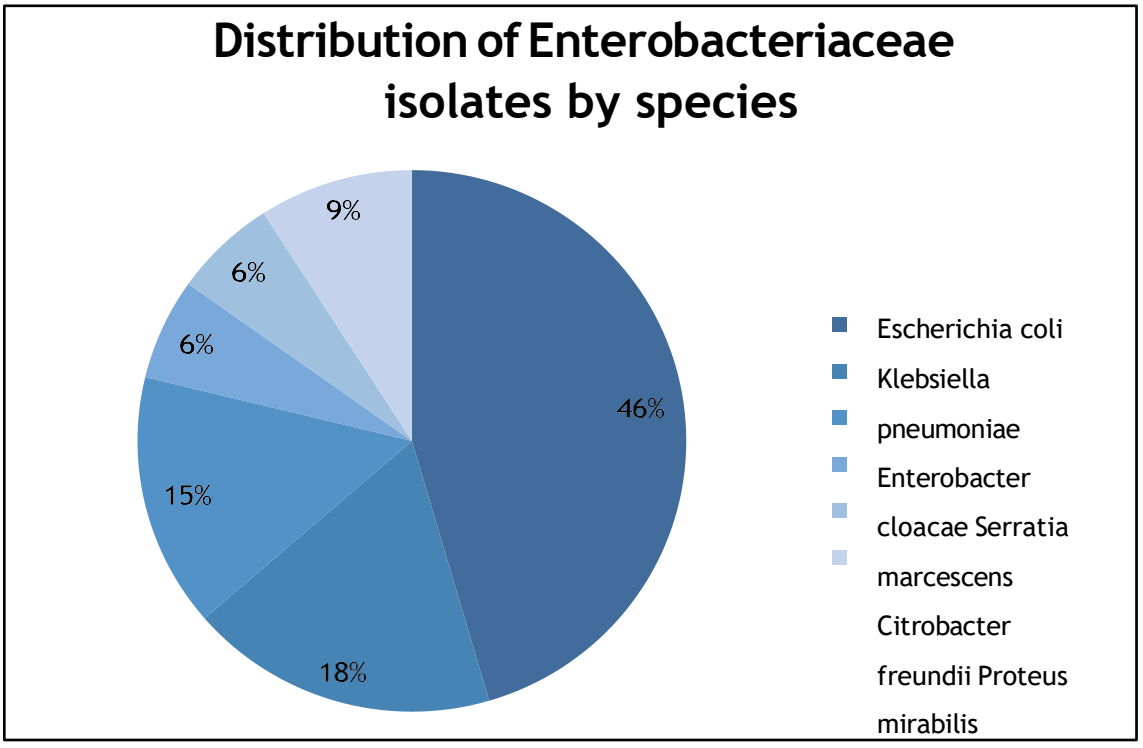

Figure 16: Distribution of Enterobacteriaceae isolates byspecies

Table 7: Results of biochemical identification of Enterobacteriaceae isolated from camel feces (n = 33)

Bacterial species	**Number of isolates**	**Percentage (%)**
Escherichia coli	15	45.45
Klebsiella pneumoniae	6	18.18
Enterobacter cloacae	5	15.15
Proteus mirabilis	3	9.09
Serratia marcescens	2	6.06
Citrobacter freundii	2	6.06
Total	**33**	**100**

These findings confirm the microbial diversity of Enterobacteriaceae present in the fecal flora of camels from the study region, with a marked predominance of *E. coli*, a species frequently regarded as an indicator of fecal contamination and an opportunistic pathogen.

The relatively high frequency of *Klebsiella pneumoniae* and *Enterobacter cloacae* isolates is notable, as these species are often implicated in opportunistic and nosocomial infections in both animals and humans.

VII.2. Results of Antibiotic Susceptibility Testing of the Isolated Enterobacteriaceae

Antimicrobial susceptibility testing was performed on the 33 identified Enterobacteriaceae isolates from the 40 camel fecal samples. The antibiotics tested represented major classes commonly employed in veterinary medicine, including β-lactams, aminoglycosides, quinolones, tetracyclines, and phenicols.

Antibiotics tested:

- Amoxicillin–clavulanic acid (AMC)
- Cefotaxime (CTX)
- Gentamicin (GEN)
- Enrofloxacin (ENR)
- Tetracycline (TET)
- Chloramphenicol (CHL)

The antibiogram results revealed overall high susceptibility rates. The most effective antimicrobial was enrofloxacin, with 100% susceptibility, followed by gentamicin (96.96%) and cefotaxime (93.93%). Resistance rates were low, particularly for amoxicillin–clavulanic acid (12.12%) and tetracycline (18.18%).

Table 8: Susceptibility of Enterobacteriaceae isolates to tested antibiotics (n = 33)

Antibiotic	Susceptible (n)	% Susceptible	Resistant (n)	% Resistant
Enrofloxacin (ENR)	33	100.00%	0	0.00%
Gentamicin (GEN)	32	96.96%	1	3.03%
Cefotaxime (CTX)	31	93.93%	2	6.06%
Amoxicillin–clavulanate (AMC)	29	87.87%	4	12.12%
Chloramphenicol (CHL)	28	84.84%	5	15.15%
Tetracycline (TET)	27	81.81%	6	18.18%

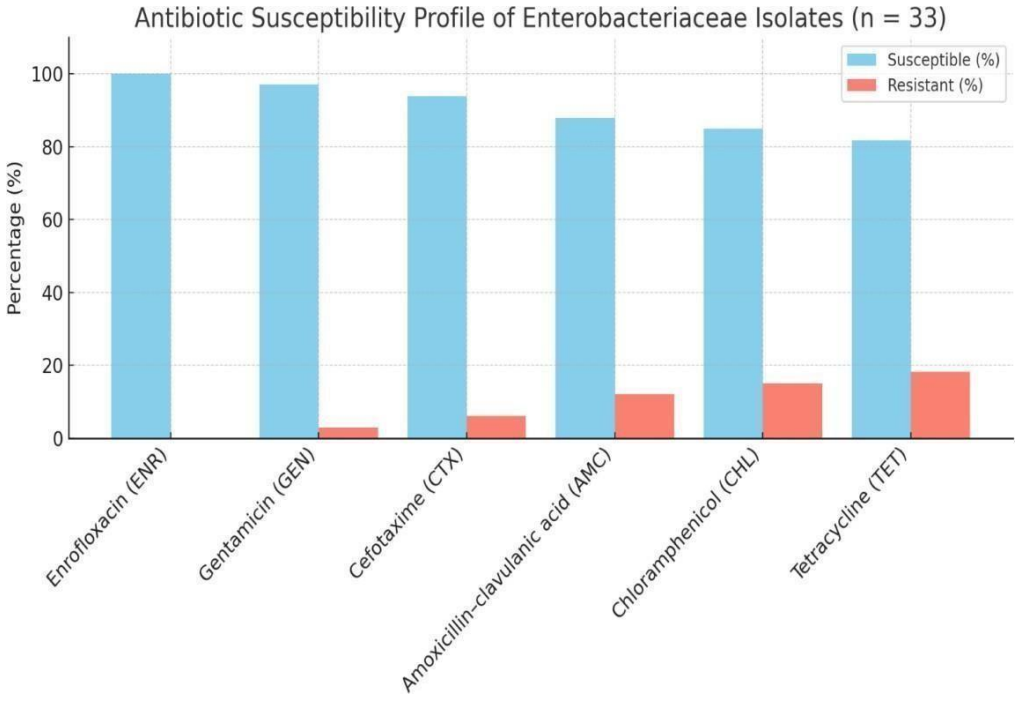

Figure 17: Overall antimicrobial susceptibility profile of Enterobacteriaceae isolates

VIII. Discussion

VIII.1. Diversity of Isolated Enterobacteriaceae

This study aimed to detect, identify, and assess the antibiotic susceptibility of Enterobacteriaceae strains isolated from camel fecal samples collected in the Ouargla region,

southern Algeria. The findings highlight relevant aspects from both microbiological and veterinary public health perspectives.

Out of 40 analyzed samples, 33 yielded Enterobacteriaceae isolates, corresponding to an isolation rate of 82.5%. The predominant species represented the typical commensal intestinal flora of camelids, including *Escherichia coli, Klebsiella spp., Enterobacter spp.,* and *Proteus spp.* These observations are consistent with prior studies on dromedaries, confirming the frequent presence of Enterobacteriaceae in the intestinal microbiota of animals raised in Saharan environments.

The predominance of *E. coli* may be attributed to its role as a sentinel bacterium, widely used as an indicator of fecal contamination and as a marker organism for antimicrobial resistance surveillance in veterinary epidemiology.

VIII.2. Antimicrobial Susceptibility Profiles

Antibiotic susceptibility testing revealed that the majority of isolates were susceptible to all tested antibiotics, with an overall low resistance profile. Enrofloxacin, a fluoroquinolone, showed 100% efficacy, followed by gentamicin (96.96%) and cefotaxime (93.93%). These promising results are particularly significant in extensive farming systems, where antimicrobial selection pressure is presumed to be minimal.

The observed high susceptibility likely reflects prudent and moderate antimicrobial use in camels in the Saharan region. Indeed, traditional livestock systems, prevalent in these areas, typically rely on occasional treatments, often under limited veterinary supervision.

Nonetheless, moderate resistance was observed to amoxicillin–clavulanic acid (12.12%), chloramphenicol (15.15%), and tetracycline (18.18%). Although these rates remain relatively low, their presence warrants attention. These antibiotics are among the most commonly used for routine infections in livestock, and their efficacy could be compromised over time if their use is not carefully managed.

These results underscore the critical need for regular surveillance of resistance profiles in commensal Enterobacteriaceae in livestock species, including camels, which are often overlooked in national antimicrobial resistance monitoring programs. Enterobacteria may serve as reservoirs for transferable resistance genes that can be acquired by pathogenic bacteria, representing a potential risk to both animal and human health.

The use of rapid identification techniques and antibiogram testing, as employed in this study, is essential to inform judicious antibiotic therapy and to combat the emergence of multidrug-resistant strains.

In conclusion, this study highlights the expected microbial diversity in the fecal flora of camels, along with high levels of susceptibility to the antibiotics tested. While the results are reassuring, they call for continued vigilance and the implementation of resistance surveillance protocols, even in extensive farming systems. The "One Health" approach—integrating animal, human, and environmental health—must also extend to the camel sector to ensure the sustainable use of antimicrobials.

Conclusion

This study highlights the emerging role of dromedaries as potential reservoirs of antimicrobial- resistant Enterobacteriaceae within the ecological and epidemiological landscape of the Algerian Sahara. Through systematic sampling, isolation, and biochemical identification, we confirmed the presence of multiple Enterobacteriaceae genera in camel fecal matter, including strains demonstrating multidrug resistance (MDR) and extended-spectrum beta-lactamase (ESBL) activity.

These findings are particularly concerning given the increasing recognition of antimicrobial resistance (AMR) as a One Health issue—where the intersection of human, animal, and environmental health facilitates the transmission and persistence of resistant bacteria. The detection of resistance to critically important antimicrobials (CIAs) used in both human and veterinary medicine underscores the urgent need for coordinated surveillance, prudent antimicrobial stewardship, and policy reform in pastoral systems.

Moreover, our work reinforces the notion that traditionally overlooked animal species—such as the dromedary—can serve as silent contributors to the AMR burden. Their close interaction with humans, shared water sources, and the growing demand for camel-derived food products elevate the risk of zoonotic transmission.

In conclusion, this research provides a foundational step toward understanding the epidemiology of AMR in camels and highlights the necessity of integrating these animals into broader national AMR monitoring programs. By promoting responsible antibiotic use and implementing targeted surveillance strategies in camel-rearing regions, we can contribute to more sustainable animal health practices and protect the efficacy of antimicrobial therapies for future generations.

60

Reference

1. Adeolu, M., Alnajar, S., Naushad, S., & Gupta, R. S. (2016). Genome-based phylogeny and taxonomy of the ‗Enterobacteriales': Proposal for *Enterobacterales* ord. nov. divided into seven families. *International Journal of Systematic and Evolutionary Microbiology, 66*(12), 5575–5599.

2. Al-Tawfiq, J. A., Laxminarayan, R., & Mendelson, M. (2017). How should we respond to the emergence of plasmid-mediated colistin resistance in humans and animals? *International Journal of Infectious Diseases, 54*, 77–84. https://doi.org/10.1016/j.ijid.2016.11.415

3. Aniashi, S. O., & Dike, O. I. (2023). Antibiotics resistance: A global health challenge.
   a. *Nigerian Journal of Microbiology.*

4. Avril, J. L., Soussy, C. J., & Lagrange, P. H. (2000). *Bactériologie Médicale* (2e éd.). Flammarion Médecine-Sciences.

5. Bakhoum, M. (2004). Étude des entérobactéries en médecine vétérinaire. *Revue de Médecine Vétérinaire, 155*(3), 161–170.

6. Bébéar, C. M., Bébéar, C., & Renaudin, H. (2006). L'identification bactérienne automatisée
   a. : systèmes API et MINI API. *Revue Française des Laboratoires* (381), 41–46.

7. Bidet, P., & Bingen, E. (2007). Caractérisation des souches de *Shigella* et *Yersinia* : particularités de culture. *Annales de Biologie Clinique, 65*(4), 393–401.

8. Bouazza, A., Belkebir, R., & Gharout-Sait, A. (2016). Étude de l'antigène commun chez les entérobactéries. *African Journal of Clinical Microbiology, 18*(2), 89–93.

9. Chakraborty, D., Debnath, F., Giri, S., Saha, S., Pyne, S., Chakraverty, R., Majumdar, A., Deb, A. K., Bhatia, R., & Dutta, S. (2024). Contribution of veterinary sector to antimicrobial resistance in One Health compendium: An insight from available Indian evidence. *Frontiers in Veterinary Science, 11*, 1411160. https://doi.org/10.3389/fvets.2024.1411160

10. Chandran, S. P., Kim, K., & Gwak, J. H. (2022). *Enterobacteriaceae*: Taxonomy, characteristics, and clinical significance. *Journal of Bacteriology Research, 14*(2), 45–53.

11. Davin-Regli, A., & Pagès, J.-M. (2015). *Enterobacter aerogenes* and *Enterobacter cloacae*; versatile bacterial pathogens confronting antibiotic treatment. *Frontiers in Microbiology, 6*, 392. https://doi.org/10.3389/fmicb.2015.00392

12. Fauchère, J. L., & Avril, J. L. (2002). *Les Entérobactéries : Aspects cliniques, biologiques et thérapeutiques*. Flammarion Médecine-Sciences.

13. Ferron, R. (1993). Antigènes d'adhésion chez les entérobactéries pathogènes animales.
   a. *Revue de Médecine Vétérinaire, 144*(5), 419–424.

14. Gadou, H. (2019). Étude biochimique des entérobactéries isolées des infections urinaires [Thèse de Pharmacie, Université d'Alger].

15. Kim, K. S., & Gadd, M. E. (2008). Role of flagellar antigen H in virulence of a. *Enterobacteriaceae*. *Microbial Pathogenesis, 45*(2), 103–109.

16. Konaré, D. (2017). Antigènes bactériens et immunologie des entérobactéries [Thèse de Médecine, Université des Sciences de la Santé, Bamako].

17. Kumar, S., Singh, A., & Joshi, R. (2023). Advances in the serotyping and classification of a. *Enterobacteriaceae*. *Microbiology Insights, 16*, 11786361231102647.

18. Liu, Y.-Y., Wang, Y., Walsh, T. R., Yi, L.-X., Zhang, R., Spencer, J., ... & Shen, J. (2016). Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. *The Lancet Infectious Diseases, 16*(2), 161–168. https://doi.org/10.1016/S1473-3099(15)00424-7

19. Lopez, A., McCabe, J., & Smyth, D. (2021). Metabolic and biochemical properties of a. *Enterobacterales*. *Clinical Microbiology Reviews, 34*(1), e00215-20.

20. Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., & Stahl, D. A. (2018). a. *Brock Biology of Microorganisms* (15th ed.). Pearson.

21. Mezzatesta, M. L., Gona, F., & Stefani, S. (2012). *Enterobacter cloacae* complex: Clinical impact and emerging antibiotic resistance. *Future Microbiology, 7*(7), 887–902. https://doi.org/10.2217/fmb.12.61

22. Murray, P. R., Rosenthal, K. S., & Pfaller, M. A. (2016). *Medical Microbiology* (9th ed.). Elsevier.

23. Nordmann, P. (2011). Infections à entérobactéries multirésistantes chez l'homme et l'animal. *Médecine et Maladies Infectieuses, 41*(9), 491–498.

24. O'Hara, C. M., Brenner, F. W., & Miller, J. M. (2000). Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clinical Microbiology Reviews, 13*(4), 534–546. https://doi.org/10.1128/CMR.13.4.534

25. Paczosa, M. K., & Mecsas, J. (2016). *Klebsiella pneumoniae*: Going on the offense with a strong defense. *Microbiology and Molecular Biology Reviews, 80*(3), 629–661. https://doi.org/10.1128/MMBR.00078-15

26. Pilet, P. E., Decosterd, F., & Delapierre, D. (1979). Caractéristiques des *Proteus* en milieux solides. *Bulletin de la Société de Pathologie Exotique, 72*(4), 319–325.

27. Willey, J. M., Sherwood, L. M., & Woolverton, C. J. (2017). *Prescott's Microbiology*
a. (10th ed.). McGraw-Hill.

28. Wilson, B. A. (2010). *Bacterial Pathogenesis: A Molecular Approach* (3rd ed.). ASM Press.

29. Wessels, M. R., Goldmann, D. A., & Kaplan, S. L. (2018). Capsular polysaccharide antigens and bacterial virulence. *Clinical Infectious Diseases, 66*(Suppl_1), S31–S37.

30. Wyres, K. L., & Holt, K. E. (2018). *Klebsiella pneumoniae* as a key trafficker of drug resistance genes from environmental to clinically important bacteria. *Current Opinion in Microbiology*, *45*, 131–139. https://doi.org/10.1016/j.mib.2018.04.004

31. Wikipedia contributors. (2023, September 10). *Avian pathogenic Escherichia coli*. *Wikipedia*. https://en.wikipedia.org/wiki/Avian_pathogenic_Escherichia_coli

32. Wikipedia contributors. (2023, November 15). *Enterobacter cloacae*. *Wikipedia*. https://en.wikipedia.org/wiki/Enterobacter_cloacae

33. Wikipedia contributors. (2023, November 15). *Proteus (bacterium)*. *Wikipedia*. https://en.wikipedia.org/wiki/Proteus_(bacterium)

34. Zhang, Z., Zhang, L., Dai, H., Zhang, H., Song, Y., An, Q., Wang, J., & Xia, Z. (2022). Multidrug-resistant *Klebsiella pneumoniae* complex from clinical dogs and cats in China: Molecular characteristics, phylogroups, and hypervirulence associated determinants. *Frontiers in Veterinary Science, 9*, 816415. https://doi.org/10.3389/fvets.2022.816415

35. Alhassan, M. Y., & Ahmad, A. A. (2025). Antimicrobial resistance in a changing climate: A One Health approach for adaptation and mitigation. *Bulletin of the National Research Centre, 49*(1). https://doi.org/10.1186/s42269-025-01318-2

36. Janda, J. M., & Abbott, S. L. (2021). The changing face of the family
a. *Enterobacteriaceae* (Order: ―*Enterobacterales*‖): New members, taxonomic issues, geographic expansion, and new diseases and disease syndromes. *Clinical Microbiology Reviews, 34*(2). https://doi.org/10.1128/cmr.00174-20

37. Sara, M., et al. (2021). Dietary risk of blaESBL producing multidrug resistant *Enterobacteriaceae* and their inhibition by *Artemisia herba-alba* and *Thymus algeriensis* essential oils. *Journal of Essential Oil Bearing Plants, 24*(3), 658–670. https://doi.org/10.1080/0972060x.2021.1937334

38. Bilal, Z., et al. (2025). Study of biological safetyof camel milk after treatment with different antibiotics. *PLOS ONE, 20*(4), e0321807. https://doi.org/10.1371/journal.pone.0321807