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Research Article

Antibiotic Resistance in Enterobacteria Isolated from Animals on Small-scale Family Farming Systems in Yopougon and Bingerville (Abidjan, Côte d'Ivoire)

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Abstract

Small-scale household livestock farming (poultry, rabbits, sheep) plays a significant role in the economy and food security of Ivorian households, but the empirical use of antibiotics in these informal farms potentially promotes the emergence and spread of resistant bacteria. This descriptive cross-sectional study, conducted at the Pasteur Institute of Côte d'Ivoire, aimed to characterize the antibiotic resistance profile of enterobacteria isolated from animals raised in family backyards in Yopougon and Bingerville. Sixty-seven rectal and cloacal swabs were collected between November 2024 and January 2025 from sheep, rabbits, and chickens. Standard bacteriological analyses were performed, and a standardized antibiotic susceptibility testing protocol was carried out in accordance with the 2023 recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM/EUCAST, 2024). Twenty-seven strains of *Enterobacteriaceae* were isolated, including *Escherichia coli* (66.7%), *Klebsiella pneumoniae* (22.2%), and *Enterobacter cloacae* (11.1%). Statistical analysis using R (RStudio) revealed a particularly high resistance rate to colistin (70.4%), followed by tetracycline and chloramphenicol (29.6% each) and nalidixic acid (22.2%). Cephalosporinase production was detected in two strains (7.4). These results reflect significant antibiotic pressure in household farming systems, particularly regarding colistin, which is used as a last resort in the treatment of severe infections caused by multidrug-resistant Gram-negative bacteria. These findings highlight the need for regulation of veterinary antibiotic use.

Keywords

Antibiotic Resistance, *Enterobacteria*, Small-scale Family Farming Farms, Abidjan District, Côte d'Ivoire

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1. Introduction

The constant increase in demand for protein worldwide poses a food security problem, particularly in low- and middle-income countries. The growth of the livestock sector, particularly non-conventional livestock (poultry, rabbits, sheep, goats, rodents, etc.), offers considerable opportunities to reduce poverty and improve food security for populations [1]. This growth in demand is sometimes accompanied by a widespread and sometimes uncontrolled use of antibiotics for curative or prophylactic purposes in family farms, where prior veterinary diagnosis remains rare and animal self-medication, from products purchased on the informal market, is often the norm rather than the exception.

This intensive use promotes the emergence of resistant bacteria, particularly within commensal *enterobacteriaceae* such as *Escherichia coli* and *Klebsiella pneumoniae*, which can then be transmitted to humans through direct contact or through the food chain [2].

According to the WHO, antimicrobial resistance is now one of the top ten global threats to public health, and its veterinary dimension is now recognized as a major driver of its spread. Globally, nearly 70% of medically important antibiotics continue to be used in animal production, a particularly high proportion in low- and middle-income countries where the intensification of livestock farming is not always accompanied by adequate veterinary oversight, which promotes the uncontrolled use of antimicrobials and increases selective pressure on commensal and pathogenic bacteria [3-6]. In Sub-Saharan Africa, this problem is aggravated by often free access to veterinary antibiotics, frequent use for prophylactic rather than curative purposes, and still limited capacity to monitor antimicrobial resistance of animal origin, especially in family and non-professionalized livestock farming systems that largely escape official statistics [7-9].

In Côte d'Ivoire, domestic poultry meat consumption is estimated at 97,000 metric tons, while local production covers only 44.6% of demand [10]. In addition to well-regulated industrial sectors, informal poultry and rabbit farming sectors persist, where chickens, rabbits, and other small ruminants are raised at the household level, with direct access to veterinary antibiotics sold over the counter in markets [8].

In addition, recent environmental studies clearly show that practices in low-resource farms generate ecotoxicological risks and promote the dissemination of resistance genes in soils and waters [11]. In this context, in order to better understand the role of family farms in the multiplication and dissemination of antibiotic resistance, it appears advisable to document the resistance profiles of isolated bacteria. Considering this, the current research was conducted on livestock from family-run farms in the Yopougon and Bingerville municipalities.

2. Materials and Methods

2.1. Site and Period of Study

This is a descriptive cross-sectional study conducted in Côte d'Ivoire from November 2024 to January 2025 in about ten family-run livestock farms located in Yopougon and Bingerville. These municipalities were selected because of the large number of industrial, domestic, and family-run livestock operations located there. Bacteriological analyses were performed at the laboratory of the National Reference Center for Antibiotic Resistance (NRC/AMR) at the Pasteur Institute of Côte d'Ivoire, Adiopodoume site.

2.2. Study Population

The study population consisted of animals raised in family yards commonly called "family farms". These were traditional chickens, hybrid-bred chickens, sheep and rabbits.

2.3. Sampling and Transport of Samples

Fecal samples were taken by rectal and cloacal swabs using sterile swabs, then stored in hemolysis tubes containing about 2 ml of physiological water. The samples collected were identified (animal species, date, municipality of collection, and biological product), and transported in a refrigerated cooler at 4°C to the laboratory of the National Reference Center for Antibiogram (NRC-ATB) of the Institute Pasteur of Côte d'Ivoire, site of Adiopodoume.

2.4. Bacteriological Isolation and Identification

The hemolysis tubes containing the swabs were incubated at 37°C for 24 hours. The next day, the samples were seeded on a medium agar with Methylene Blue Eosine (EMB) by the streak method. Three to five identical suspicious colonies were isolated and identified by conventional biochemical tests distinguishing *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*.

2.5. Sample Storage

The samples and isolated bacterial strains were stored at the Biological Resources Center (CeReB) in a freezer at -80°C, in 20% glycerol in cryotubes, according to validated methods for fecal matrices [12]. Before performing the antibiotic susceptibility testing, the strains were recovered under aseptic conditions, thawed, and then inoculated onto selective nutrient agar media to ensure their viability and purity. The isolates were then tested for antimicrobial susceptibility and phenotypically characterized according CA-SFM/EUCAST procedures.

2.6. Antimicrobial Susceptibility Test

The Antimicrobial susceptibility test of the isolates was determined using the Mueller-Hinton agar diffusion method according to the Kirby-Bauer method, using an inoculum adjusted to 0.5 McFarland. The panel of antibiotics tested consisted of Bio-Rad discs: amoxicillin + clavulanic acid (75 µg), cefepime (5 µg), cefixime (5 µg), cefoxitin (30 µg), gentamicin (10 µg), kanamycin (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), nalidixic acid (30 µg), enrofloxacin (5 µg), colistin (10 µg), and trimethoprim-sulfamethoxazole (1.25 µg/25.75 µg). Inhibition zone diameters were measured using the ADAGIO® automated system (Bio-Rad), and the categories "susceptible," "resistant," or "intermediate" were assigned based on the critical values recommended by the CA-SFM. Quality control was performed using the reference strain *Escherichia coli* ATCC 25922.

2.7. Statistical Analysis

The data were entered and then analyzed using R (version 4.3.3, RStudio environment). Population sizes and proportions were calculated for the distribution of sampled animal species, isolated bacterial species, and antibiotic resistance rates. The significance of heterogeneity in the distribution of isolated bacterial species was tested by a Chi-square test. The rate of resistance to colistin was compared to a reference threshold of 10% by a unilateral exact binomial test (function binom. test), in order to objectify its significantly high character. The graphical representations were produced with the ggplot 2 package. The threshold of significance was $p < 0.05$.

3. Results and Discussion

3.1. Results

A total of 67 samples were collected from hybrid chickens (n = 27; 40.3%), rabbits (n = 19; 28.4%), local chickens (n = 16; 23.9%) and sheep (n = 5; 7.5%).

3.1.1. Isolated Bacterial Species

27 strains of *enterobacteriaceae* from the 67 samples collected were identified, comprising 18 strains of *Escherichia coli* (66.7%), 6 strains of *Klebsiella pneumoniae* (22.2%), and 3 strains of *Enterobacter cloacae* (11.1%) (Figure 1) ($\chi^2 = 14.0$; ddl = 2; $p < 0.001$).

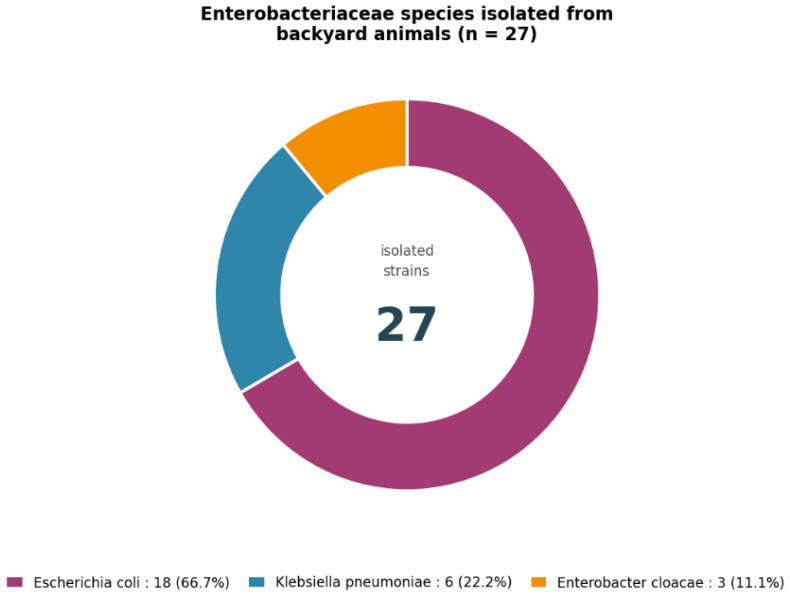


Figure 1. *Ring plot showing the distribution of Enterobacteriaceae species isolated from backyard animals, with the total number shown in the center (n = 27).*

3.1.2. Antibiotic Resistance Profile

The susceptibility test revealed a particularly high rate of resistance to colistin (70.4%; 19/27), which was significantly higher than a reference threshold of 10% (one-sided exact binomial test, $p < 0.001$; 95% CI: 52.9–100%). More moderate rates were observed for tetracycline (29.6%) and chloramphenicol (29.6%), as well as for nalidixic acid (22.2%) (Figure 2).

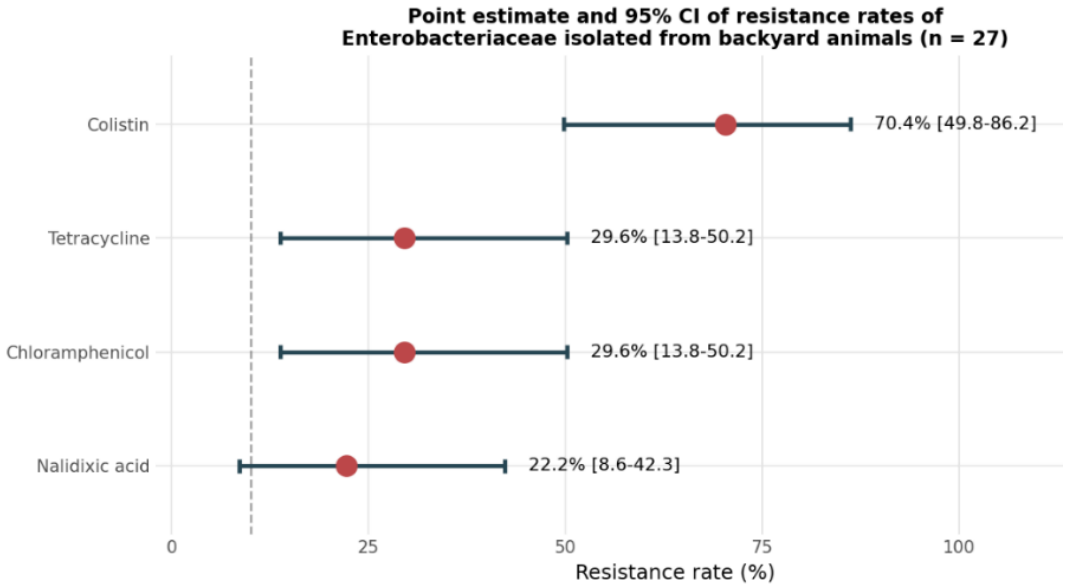


Figure 2. *Estimation points and 95% confidence interval for the resistance rates of enterobacteriaceae isolated from animals on family farms by antibiotic (n = 27).*

Phenotypically, 25 strains (92.6%) exhibited a wild-type phenotype, while 2 strains (7.4%) exhibited a resistance phenotype (*Klebsiella pneumoniae* and *Enterobacter cloacae*) through the production of cephalosporinase, conferring resistance to all cephalosporins.

3.2. Discussion

The predominance of *Escherichia coli* (66.7%) among the isolated enterobacteria is consistent with its status as the dominant commensal bacterium in the gut microbiota of mammals and birds. However, the very high rate of colistin resistance observed (70.4%) is a particularly concerning finding in family-run farms. This result is consistent with data from recent studies confirming the widespread prevalence of *Escherichia coli* strains carrying plasmid-mediated resistance genes such as *mcr*-1 [13, 14]. Indeed, colistin remains a last-resort antibiotic used in human medicine to treat severe infections caused by multidrug-resistant Gram-negative bacteria. Its intensive utilization in veterinary medicine has facilitated to the emergence and global dissemination of plasmid-mediated resistance genes that can be transmitted to humans, particularly *mcr-1* demonstrate that the global spread of *mcr* genes is a major public health concern [15-19].

With regard to tetracycline and chloramphenicol, this study shows resistance rates of 29.6 % each. This situation is cause for concern. A few decades ago, these drugs were cornerstones of treatment in livestock farms in west Africa due to their low cost and wide availability [8]. However, despite advances in antibiotic development, tetracycline remains the antibiotic most frequently associated with resistance (43 % of reported cases) in African livestock farms [20].

The detection of cephalosporin resistance in two strains (7.4%), although a minority, warrants special attention. Indeed, when genes encoding cephalosporinases are carried by plasmids, they can be transmitted from bacteria in animals to humans, thereby contributing to the spread of antibiotic resistance [21, 22].

4. Limitations of the Study

The limited sample size used in this study does not allow for an accurate estimate of the prevalence of antibiotic resistance in family-run farms.

5. Conclusion

This study showed that *Enterobacteriaceae* isolated from animals raised on family farming systems in Yopougon and Bingerville exhibited high levels of resistance to several antibiotics, including colistin, tetracycline, and chloramphenicol. In addition, a resistance to third-generation cephalosporins was observed in two strains. These results highlight the need for integrated surveillance strategies that takes into account even informal livestock production systems, which are often regarded as having limited exposure to antimicrobial resistance.

Abbreviations

CA-SFM/EUCAST	Comite de l'Antibiogramme de la Societe Française de Microbiologie (French National/ European Committee on Antimicrobial Susceptibility Testing)
NRC/AMR	National Reference Center for Antimicrobial Resistance
ATB	Antibiogram
CeReB	Biological Resources Center
ATCC	American Type Culture Collection
WHO	World Health Organization

Author Contributions

Beudje Felicite: Conceptualization, Resources, Investigation, Data curation, Writing – original draft, Methodology

Ettien Marcelle Monnet: Formal Analysis, Investigation, Methodology, Writing – review & editing

Odegue Kpadraux Danielle: Formal Analysis, Investigation, Methodology, Writing – review & editing

N'Gbesso N'Gbesso Jean Paul: Formal Analysis, Software, Data curation

Afran Sidje Arlette: Data curation**,** Writing – review & editing, Software

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Konan Fernique: Supervision, Visualization

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Guessennd Nathalie: Validation, Supervision, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interests.

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