International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Antibiotic resistance in Gram-negative bacilli isolated from livestock farms in Gabon

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International Journal of Science and Research Archive, 2025, 14(01), 1764-1773

Publication history: Received on 07 December 2024; revised on 23 January 2025; accepted on 26 January 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.14.1.0146

Abstract

The aim of this study was to determine the prevalence of antibiotic resistance among Gram-negative bacilli on livestock farms. A total of 356 faecal samples from farms in 9 cities across 6 provinces were collected. Isolates obtained after culture and isolation were identified using the API 20E gallery. Following culture, 51.4% of the samples yielded colonies. Identifications revealed Escherichia (30.0%), followed by *Pseudomonas* (26.2%), *Serratia* (17.5%), and Enterobacter (6.56%), among others. Antibiotic susceptibility testing was conducted using the disc diffusion method. Susceptibility tests indicated resistance to *Ampicillin* (68.3%), *Amoxicillin + Clavulanic Acid* (66.7%), *Cefotaxime* (65.6%), *Cefepime* (60.1%), *Aztreonam* (59%), and *Ertapenem* (49.2%). Maximum resistance to *Ceftazidime* (77.0%) was observed, particularly in Escherichia, Pseudomonas, Serratia, and Enterobacter, posing a public health problem and a threat to the health of these Populations. All identified isolates exhibited antibiotic resistance at least to three or more antimicrobial family of antibiotics, and were multidrug resistance (MDR) bacteria. Some of them are associated with *ESβL* (7.1%). Given that MDR bacteria were isolate from farm animals that are intended for human consumption, there is a significant risk of transmitting MDR strains to consumers, posing a substantial threat to human health.

Keywords: Multidrug-Resistant Bacteria; Antibiotic; Farm Animals; Extended-Spectrum β-Lactamase; Gabon

1. Introduction

Livestock farming in Gabon is characterized by a numerically limited and diversified herd, low productivity, and a dominant extensive farming system for domestic ruminants (cattle, sheep, goats) and monogastric animals (poultry and pigs) [1]. This type of farming inadequately meets the animal protein needs of the Gabonese population [2]. However, demographic growth and changes in dietary habits, particularly urbanization, lead to an increased demand for animal proteins in both rural and urban settings. The proximity to urban demand provides an interesting opportunity for producers to sell raw products quickly for short-distance marketing [3]. Urban farming systems significantly contribute to meeting the increased demand for animal products in cities [2]. Nevertheless, the use of antimicrobials, particularly antibiotics, in animal production is becoming a growing concern due to the selective pressure exerted by these molecules, potentially impacting commensal bacteria [4]. This impact may manifest as the development of antimicrobial resistance and the transfer of resistance genes to pathogenic bacteria [5]. These resistant bacteria may enter the food chain, contaminating humans and the environment [6], leading to the emergence of antibiotic resistance or multidrug resistance (MDR). Some of these MDR bacteria present challenges for medical and veterinary treatment [7].

Antibiotic resistance has been a significant scientific and political concern for the last three decades [8]. Studies have shown that one risk factor for acquiring MDR bacteria, such as Extended-Spectrum β-Lactamase (ESβL)-producing

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strains, is the overuse of antibiotics in poultry and pig production [9]. However, antibiotic consumption in livestock is poorly understood in Gabon [10]. The World Health Organization (WHO) emphasizes the need to enhance global surveillance of antibiotic resistance. Hence this study focuses on antibiotic resistance in Gram-negative bacilli (GNB) from livestock farms, aiming to conduct a comprehensive screening for various resistance mechanisms, including ESβL production.

2. Material and methods

2.1. Ethics approval and consent to participate

This research was conducted in Gabon through a collaborative effort between IRET and the University of Science and Technology of Masuku (USTM) under Research Agreement No. 160/2020/MESRSTTENCFC/USTM/FS/Dn, dated August 7, 2020. This study was approved by the Gabonese Ministry of Agriculture, Livestock, Fisheries and Rural Development (Livestock Department authorization n0052/SG/DGE). All samples taken from livestock were collected with the verbal consent of the farm managers.

2.2. Study Setting and sampling

The sample analysis took place in the Microbiology and Molecular Biology Laboratory of the Institute of Research in Tropical Ecology (IRET) at CENAREST.

2.3. Study Type and Duration

This prospective study occurred over 5 months from August to December 2020.

2.4. Sampling

Fecal samples from animals were collected in 9 cities (Libreville, Oyem, Franceville, Moanda, Mouila, Koulamoutou, Lastoursville, Mounana, Tchibanga,), spanning 6 provinces (Estuaire, Haut-Ogooué, Ogooué-Lolo, Ngounié, Nyanga) during two main periods: September 2020 and December 2020. After collecting animal faeces (poultry, pigs, cattle, and sheep) in sterile 100 ml tubes, each sample was stored in a plastic bag, protected from sunlight, and transported to the IRET laboratory. For better preservation, each sample was stored in duplicate in a PBS/glycerol mixture (70/30%) in a 2 ml cryotube. Cryotubes were stored at -20°C, while 100 ml tubes were kept in the refrigerator at +4°C in the IRET bacteriology laboratory.

2.5. Laboratory Bacteriological Analyses

2.5.1. Culture, Isolation, and Identification of Colonies

Fecal samples were brought to room temperature after removal from the refrigerator, and 100 µL of the Phosphate Buffers sallin (PBS)/glycerol mixture (70%/30%) were taken, deposited into a 2 ml tube, and centrifuged at 14,000 rpm. After centrifugation, the supernatant was discarded, and each bacterial pellet was taken using a sterile 1 µl plastic loop, then streaked over the entire surface of MacConkey Agar (MCA) (BioMérieux, France) using the streak plate method and incubated at 37°C for 24 hours. Each colony obtained after incubation was identified using the Api 20E gallery (BioMérieux, France). The reading, after adding reagents, was performed using the ApiWeb software (BioMérieux, France).

2.5.2. Antibiotic Susceptibility Testing

A preliminary test to select ESβL-producing bacteria involved replicating all colonies grown on MCA supplemented with 2 mg/L of cefotaxime (CTX) (MCA/CTX). Compared to non-supplemented MCA, the MCA/CTX combination significantly increases the detection of beta-lactam resistance [11]. ESβL production was tested using the double-disc synergy test (DDST). All beta-lactams were tested simultaneously on the same antibiogram to determine acquired or intrinsic phenotypes. Antibiotic susceptibility tests were performed using the disc diffusion method on Muller Hinton agar (BioMérieux, France) following Bauer et al.'s protocol [12]. The interpretation was based on measuring the diameter of inhibition zones using a digital caliper (ATORN KEEPTRONIC, Germany) and interpreted according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (version 7.1). The following antibiotics, commonly used for treating bacterial infections in local clinics, were tested: Nalidixic Acid NAL 30 µg, Amikacin AMK 30 µg, Amoxicillin+Clavulanic acid AMC 30 µg, Ampicillin AMP 10 µg, Aztreonam ATM 30 µg, Cefepime FEP 30 µg, Cefotaxime CTX 30 µg, Cefpodoxime CPD 10 µg, Ceftazidime CAZ 10 µg, Chloramphenicol CHL 30 µg, Ciprofloxacin CIP 5 µg, Ertapenem ETP 10 µg, Gentamicin GEN 10 µg, Imipenem IPM 10 µg, Nitrofurantoin NIT 300 µg,

Norfloxacin NOR 10 µg, Ofloxacin OFX 5 µg, Tetracycline TET 30 µg, Tobramycin TOB 10 µg and Sulfamethoxazole/Trimethoprim SXT 23.7 µg

3. Results

3.1. Sampling

A total of 356 livestock faecal samples (goats, cattle, pigs, and chickens) collected from farms in 7 Gabonese cities were analyzed. Out of the 356 cultured faecal samples, bacterial colonies grew for 183 samples.

3.2. Distribution of Samples by Animal Type

Among the 183 positive samples, pigs, cattle, chickens, and goats accounted for 90 samples (49.2%), 47 samples (25.7%), 30 samples (16.4%), and 16 samples (8.7%) respectively (Figure 1).

3.2.1. Identification of Gram-Negative Bacilli (GNB) (Figure 1)

From these samples, 183 GNB were isolated and identified. For each positive culture, the most representative colony based on morphology and colour was chosen for identification. Among the identified GNB, there were isolates of *Escherichia coli* (55, 30.0%), *Pseudomonas* spp (48, 26.2%) (*Pseudomonas oryzihabitans* (36, 19.7%), *Pseudomonas luteola* (7, 3.8%), *Pseudomonas aeruginosa* (3, 6.2%), *Pseudomonas spp* (2, 1.1%)), *Serratia* spp (32, 17.5%) (*Serratia odirifera* (12, 6.5%), *Serratia phymuthica* (7, 3.8%), *Serratia* spp (5, 2.7%), *Serratia ficaria* (5, 2.7%), *Serratia liquefaciens* (3, 1.6%), *Serratia marcescens* (1, 0.5%)), *Enterobacter* spp (12, 6.6%) (*Enterobacter sakazaki* (5, 2.7%), *Enterobacter cloacae* (3, 1.6%), *Enterobacter* spp (3, 1.6%), *Enterobacter amnigenus* (1, 0.5%)), *Pantoea* spp (8, 4.4%), *Klebsiella* (6, 3.3%) (*Klebsiella oxytoca* (5, 2.7), *Klebsiella pneumoniae* (1, 0.5%)), *Citrobacter freundii* (4, 2.2%), *Proteus* spp (4, 2.2%) (*Proteus mirabilis* (2, 1.1%), *Proteus vulgaris* (2, 1.1%)), *Salmonella* (4, 2.2%) (*Salmonella choleraesuis* (3, 1.6%), *Salmonella* spp (1, 0.5%)), *Stenotrophomonas maltophilia* (1.6%), *Acinetobacter* spp (2, 1.1%), *Pasteurella pneumotropica* (2, 1.1%), and *Vibrio milicus* (1, 0.5%), *Aeromonas hydrophila* (1, 0.5%), and *Kluyvera* spp (1, 0.5%).

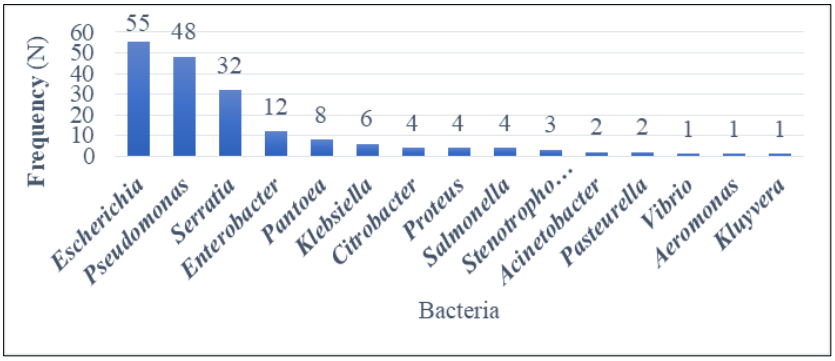

Figure 1 Prevalence of Identified Gram-Negative Bacilli (GNB)

3.3. Distribution of GNB by Animal (Figure 2).

3.3.1. Chickens

In chickens, isolates were obtained were *Escherichia coli* (6, 20.0%), *Pseudomonas oryzihabitans* (6, 20,0%), *Serratia liquefaciens* (2, 6.7%), *Serratia phymuthica* (2, 6.7%), *Serratia* spp (2, 6.7%), *Pseudomonas* spp (2, 6.7%), *Citrobacter freundii* (2, 6.7%), *Aeromonas hydrophila* (1, 3.3%), *Enterobacter amnigenus* (1, 3.3%), *Stenotrophomonas maltophilia* (1, 3.3%), *Enterobacter cloacae* (1, 3.3%), *Enterobacter sakazakii* (1, 3.3%), *Enterobacter* spp (1, 3.3%), *Salmonella choleraesuis* (1, 3.3%), and *Pantoea* spp (1, 3.3%).

3.3.2. Pigs

The following species were obtained from pigs: *Escherichia coli* (38, 42.2%), *Pseudomonas oryzihabitans* (17, 18.9%), *Serratia odorifera* (7, 7.8%), *Klebsiella oxytoca* (3, 3.3%), *Acinetobacter* spp (2, 2.2%), *Citrobacter freundii* (2, 2.2%), *Enterobacter cloacae* (2, 2.2%), *Enterobacter* spp (2, 2.2%), *Proteus mirabilis* (2, 2.2%), *Serratia ficaria* (2, 2.2%), *Enterobacter Sakazakii* (1, 1.1%), *Kluyvera* spp (1, 1.1%), *Proteus vulgaris* (1, 1.1%), *Pseudomonas aeruginosa* (1, 1.1%),

Pseudomonas luteola (1, 1.1%), *Salmonella choleraesuis* (1, 1.1%), *Salmonella* spp (1, 1.1%), *Serratia liquefaciens* (1, 1.1%), *S. marcesceus* (1, 1.1%), *Serratia* spp (1, 1.1%), and *Vibrio milicus* (1, 1.1%).

3.3.3. Goats

The following species were obtained from goats: *Pseudomonas oryzihabitans* (4, 25.0%), *Escherichia coli* (3, 18.7%), *Klebsiella oxytoca* (2, 12.5%), *Pasteurella pneumotropica* (2, 12.5%), *Serratia ficaria* (2, 12.5%), *Pseudomonas aeruginosa* (1, 6.2%), *Serratia odorifera* (1, 6.2%), and *Serratia phymuthica* (1, 6.2%).

3.3.4. Cattle

The following species were obtained from cattle: *Pseudomonas oryzihabitans* (9, 19.1%), *Escherichia coli* (8, 17.0%), *Pantoea* spp (7, 14.9%), *Pseudomonas luteola* (4, 8.5%), *Serratia odorifera* (4, 8.5%), *Serratia phymuthica* (4, 8.5%), *Enterobacter sakazakii* (3, 6.4%), *Serratia* spp (2, 4.2%), *Serratia maltophilia* (2, 4.2%), *Klebsiella pneumoniae* (1, 2.1%), *Pseudmonas aeruginosa* (1, 2.1%), *Salmonella choleraesuis* (1, 2.1%), and *Serratia ficaria* (1, 2.1%).

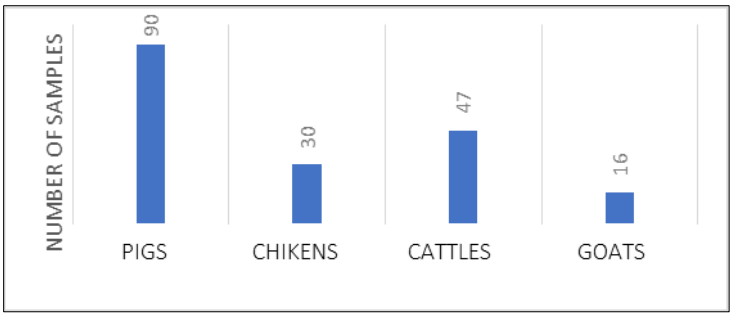

Figure 2 Distribution of Positive Bacterial Culture Samples by Animal

3.4. Antibiotic Susceptibility

3.4.1. Resistance Phenotypes

During this study, 20 antibiotics were tested on the 183 isolated and identified GNB. The GNB were classified as susceptible (S), intermediate (I), or resistant (R) to antibiotics based on their inhibition zone diameters interpreted according to Clinical and Laboratory Standard Institute (CLSI 2015) recommendations. The antibiotic susceptibility test revealed several resistance profiles. Table 1 shows that antibiotics belonging to the β-Lactam family (especially 3rd generation Cephalosporins (CAZ (141, 77.0%), CTX (120, 65.6%), FEP (110, 60.1%), CPD (77, 42.1%)), Aminopenicillins (AMP (125, 68.3%), AMC (122, 66.7%)), Monobactam (ATM (108, 59.1%)), and Carbapenems (ETP (90, 49.2%)) had the highest resistance prevalences. They were followed by Tetracyclines (TET (61, 33.3%)), β-Lactam (IMP (44, 24.0%)), and Nitrofuran (NIT (44, 24.0%)), Sulfonamides (SXT (34, 18.6%)), Aminoglycosides (TOB (30, 16.4%), AMK (29, 15.8%), and GEN (18, 9.8%)), Phenicols (CHL (29, 15.8%)), and Quinolones/Fluoroquinolones (NAL (50, 27.3%), CIP (25, 13.6%), OFX (18, 9.8%), and NOR (17, 9.3%)).

In the Table bacteria showed 50% resistance to 30% (6/20) of the tested antibiotics while these was a 45% sentivity rate to the same 30% tested antibiotics. Furthermore, antibiotics with a sensitivity greater than 70.0% (14/20), such as AMK (144, 78.6%), CHL (129, 70.5%), GEN (139, 75.9%), TOB (131, 71.6%), CIP (133, 72.7%), OFX (147, 80.3%), SXT (132, 72.1%), and NOR (154, 84.1%), mainly belong to Aminoglycosides, Quinolones/Fluoroquinolones, Sulfonamides, and Phenicols, which are different from beta-lactams. It is also important to note that sensitivity to carbapenems (IMP) was high.

3.4.2. Antibiotic Resistance by Localities

The antibiotic susceptibility test revealed various resistance profiles to antibiotics in the sampled localities. This antibiotic resistance profile according to sample site can be observed in Figure 7. The distribution is not uniform, as some cities show high antibiotic resistance, while others have lower resistance.

3.4.3. Distribution of Most Resistant Antibiotics by Locality (Cities) (Figure 3)

- **Libreville :** Isolates exhibited resistance to AMP (100%) and AMC (100%), CAZ (87.5%), CTX (75.0%), ETP (75.0%), FEP (62.5%), ATM (50.0%). While isolates presented with low resistance to NIT (37.5%), NAL

(37.5%), SXT (25.0%), CHL (25.0%), OFX, NOR, TET, CIP, IPM, CPD (12.5%). GEN (0%), AMK and TOB showed no resistance.

- **Oyem :** Isolates exhibited resistance respectively to AMP (89.8%), followed by ATM (77.9%), CAZ (76.3%), AMC, CTX, and FEP, all with a resistance of 72.9%, ETP (50.8%), CPD (47.5%), TET (44.1%), NAL (30.5%), IPM (22%), NIT, SXT, AMK, CIP (20.3%), GEN, TOB, CHL (18.6%), NOR (15.3%), and OFX (13.6%). Beta-lactams, especially aminopenicillins (AMP, AMC), cephalosporins (CAZ, FEP, CTX), monobactams (ATM), carbapenems (ERT), showed the highest resistance.
- **Franceville :** Isolates exhibited resistance to CAZ and AMP at 83.3%, AMC (77.8%), ATM and FEP at 72.2%, CTX (66.7%), TET (55.6%), CPD, NAL (50.0%). Also low resistance were observed for NIT, CHL (38.9%), IPM, AMK (33.3%), ETP, TOB (22.2%), GEN, NOR, OFX, SXT (16.7%), CIP (11.1%). Resistance were highest against Third-generation cephalosporins (C3G) followed by penicillin and carbapenem. Beta-lactams were the most resistant antibiotic family in Franceville.
- **Moanda :** Isolates exhibited resistance to CAZ (87.5%), AMP, ATM, and ETP (75.0%), AMC and FEP (62.5%), CTX, TET, and NIT (50.0%). Low resistance were observed for NAL (37.5%), CHL, AMK, TOB, SXT (25.0%), and CPD (12.5%). IPM, CIP, GEN, NOR, and OFX showed no resistance. β-lactams were the most used antibiotics, leading to resistance. However, these bacteria were more sensitive to fluoroquinolones, nitrofurans, and tetracycline.
- **Mouila :** Resistance were greatest against CAZ and ETP (66.7%), followed by CTX, AMP with respective resistances of 59.3%, 55.6%, ATM and CPD (51.8%), FEP, AMC (37.0%), NAL (22.2%), TOB, SXT, NIT (14.8%), IPM, CIP, TET, AMK, OFX (11.1%), CHL (7.4%), GEN, NOR (3.7%). In this city, third-generation cephalosporins, carbapenems, penicillins, and monobactams recorded high resistance, while sulfonamides, nitrofurans, aminoglycosides, and fluoroquinolones recorded lower resistance frequencies.
- **Koulamoutou :** Bacteria were resistant to only 5 antibiotics, that is AMC (87.5%), CAZ and AMP (75.0%) and CTX, IPM (50.0%). Following were FEP, CPD (37.5%), ATM, AMK, SXT (25.0%), ETP, NAL, TOB (12.5%). There were no resistance against CHL, CIP, TET, GEN, NOR, OFX, and NIT. β-lactams were the most used, leading to resistance. However, these bacteria were more sensitive to fluoroquinolones, nitrofurans, and tetracycline.
- **Lastourville :** Identified isolates showed higher resistance to CAZ and AMC (91.7%), TET (66.7%), CTX (58.3%), and ETP (50%), and lower resistance to FEP (41.7%), CPD, IPM, NAL, CIP, SXT, AMP (33.3%), NIT, ATM (25%), TOB (16.7%), OFX (8.3%). There were no resistance against CHL, AMK, NOR, and GEN.
- **Mounana :** Bacterial isolates presented higher resistance to CAZ (85.7%), FEP, AMC (80.9%), and CTX (71.4%), CPD (52.4%); lower resistance to ATM (42.8%), ETP, IPM, AMP (38.1%), TET (33.3%), TOB, SXT (14.3%), NAL (9.5%), AMK, NOR, OFX (4.7%); no resistance to CHL, CIP, and GEN. In Mounana, β-lactams (cephalosporins and penicillins) were the families against which isolates were resistant. However, these isolates were sensitive to quinolones/fluoroquinolones, aminoglycosides, and phenicols.
- **Tchibanga :** Resistance was higher to CAZ (63.6%), CTX (59.1%), ATM, and ETP (50.0%), AMP (45.5%), and FEP (40.9%) ; lower resistance to AMC (31.8%), CPD (27.3%), IPM (22.7%), CHL, NAL, AMK (18.2%), CIP, GEN, TOB, NIT (13.6%), TET, SXT (9.1%), OFX (4.5%) ; and no resistance to NOR.

In conclusion, the cities with the highest overall resistance, in descending order, were Franceville (43.9%), Oyem (41.2%), Libreville (37.5%), followed by Moanda (36.9%), Lastoursville (34.9%), Mounana (30.6%), Koulamoutou (26.2%), Mouila (25,2) and Tchibanga (23.1%). However, there was no statistical difference between these different resistance prevalences (Power_divergence: statistic=11.735074380165289, pvalue=0.163421376489747 17).

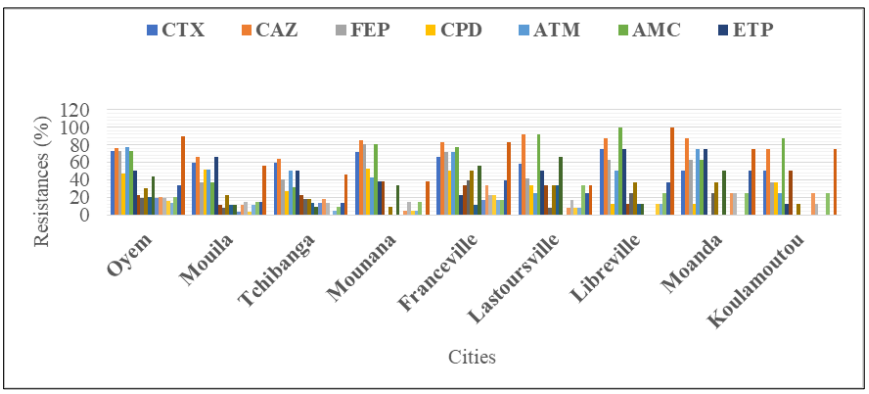

Figure 3 Prevalence of Antibiotics in Sampled Cities

3.5. Antibiotic Resistance by Animals

3.5.1. In Pigs

The highest resistances (≥ 50%) were observed for CAZ (85.6%), AMC (81.1%), FEP (76.7%), AMP (76.7%), CTX (72.2%), ATM (72.2%). The lowest resistances were for CPD (45.6%), ETP (42.2%), TET (38.9%), NIT (27.8%), IMP (25.6%), NAL (24.4%), CHL (21.2%), TOB (14.4%), AMK (14.4%), SXT (14.4%), NOR (12.2%), GEN (11.1%), OFX (10%), CIP (8.9%). Conversely, the highest sensitivities were for CPD (54.4%), ETP (57.8%), TET (61.1%), NIT (72.2%), IMP (74.4%), NAL (75.6%), CHL (78.8%), TOB (85.6%), AMK (85.6%), SXT (85.6%), NOR (87.8%), GEN (88.9%), OFX (90.0%), CIP (91.1%). The lowest sensitivities were for CAZ (14.4%), AMC (18.9%), FEP (23.3%), AMP (23.3%), CTX (27.8%), and ATM (27.8%).

3.5.2. In Chickens

The highest resistances (≥ 50%) were observed for CAZ (70.0%), AMC (66.7%), AMP (63.3%), CTX (56.7%), ETP (56.7%). The lowest resistances were for TET (53.3%), FEP (46.7%), NAL (46.7%), ATM (40.0%), CPD (40.0%), CIP (36.7%), IMP (33.3%), SXT (33.3%), NIT (30%), AMK (26.7%), TOB (23.3%), OFX (16.7%), NOR (16.7%), GEN (13.3%), and CHL (10.0%). The highest sensitivities were for FEP (53.3%), NAL (53.3%), ATM (60.0%), CPD (60.0%), CIP (63.3%), IMP (66.7%), SXT (66.7%), NIT (70.0%), AMK (73.3%), TOB (76.7%), OFX (83.3%), NOR (83.3%), GEN (86.7%), and CHL (90.0%) ; the lowest were for CAZ (30.0%), AMC (33.3%), AMP (36.7%), CTX (43.3%), ETP (43.3%), TET (46.7%).

3.5.3. In Cattle

The highest resistances (≥ 50%) were observed for CAZ (63.8%), CTX (59.6%), AMP (57.4%), and ETP (57.4%) ; low for ATM (48.9%), CPD (40.4%), FEP (38.3%), AMC (38.3%), NAL (21.3%), IMP (21.3%), SXT (17.0%), TOB (17.0%), NIT (14.9%), AMK (14.9%), CIP (12.8%), CHL (10.6%), TET (8.5%), OFX (8.5%), GEN (4.2%), and NOR (2.1%). Conversely, the highest sensitivities were for ATM (51.1%), CPD (59.6%), FEP (61.7%), AMC (61.7%), NAL (78.7%), IMP (78.7%), SXT (83.0%), TOB (83.0%), NIT (85.1%), AMK (85.1%), CIP (87.2%), CHL (89.4%), TET (91.5%), OFX (91.5%), GEN (95.8%), and NOR (97.9%) ; low for CAZ (36.7%), CTX (40.4%), AMP (42.6%), and ETP (42.6%).

3.5.4. In Goats

The highest resistances (≥ 50%) were observed for CAZ (81.2%), AMC (68.7%), CTX (62.5%), AMP (62.5%), ETP (50%), ATM (50.0%) ; low for TET (37.5%), CPD (31.2%), NAL (25.0%), SXT (18.7%), NIT (18.7%), TOB (12.5), GEN (12.5%), CHL (12.5%), AMK (6.2%), and IMP (6.2%). Conversely, the highest sensitivities were for IMP (93.8%), AMK (93.8%), CHL (87.5%), GEN (87.5%), TOB (87.5), NIT (81.3%), SXT (81.3%), NAL (75%), CPD (62.5%), TET (62.5%) and ATM (50.0%), ETP (50.0%) ; low for CAZ (18.8%), AMC (31.3%), CTX (37.5%), AMP (37.5%).

Overall, bacteria showed higher resistance against β-lactams, specifically third generation of cephalosporin (3GC) and AMC. However, bacteria isolated from all farm animals seem more sensitive to tetracycline, carbapenem (imipenem, ertapenem), quinolones/fluoroquinolones, and sulfonamides, although this depends on the animal and locality (Table 1).

Table 1 Overall Antibiotic Resistance Phenotypes

Antibiotics	**Resistant**		**Intermédiate**		**Sensitive**	
	Number	**%**	**Number**	**%**	**Number**	**%**
Amoxicillin + Clavulanic Acid (AMC)	122	66.7	16	8.7	45	24.6
Amikacin (AMK)	29	15.8	15	8.2	139	75.9
Cefotaxime (CTX)	120	65.6	11	6.0	52	28.4
Ceftazidime (CAZ)	141	77.0	3	1.6	39	21.3
Cefpodoxime (CPD)	77	42.1	29	15.8	77	42.1
Cefepime (FEP)	110	60.1	5	2.7	68	37.1
Aztreonam (ATM)	108	59.0	34	18.6	41	22.4

Imipenem (IMP)	44	24.0	21	11.5	118	64.5
Ertapenem (ETP)	90	49.2	33	18.0	60	32.8
Chloramphenicol (CHL)	29	15.8	25	13.7	129	70.5
Gentamicin (GEN)	18	9.8	22	12.0	143	78.1
Nitrofurantoin (NIT)	44	24.0	30	16.4	109	59.6
Tetracycline (TET)	61	33.3	12	6.6	110	60.1
Tobramycin (TOB)	30	16.4	22	12.0	131	71.6
Nalidixic Acid (NAL)	50	27.3	57	31.1	75	41.0
Ciprofloxacin (CIP)	25	13.7	25	13.7	133	72.7
Ofloxacin (OFX)	18	9.8	18	9.8	147	80.3
Sulfamethoxazole/Trimethoprim (SXT)	34	18.6	17	9.3	132	72.1
Ampicillin (AMP)	125	68.3	8	4.3	50	27.3
Norfloxacin (NOR)	17	9.3	12	6.6	154	84.1

3.6. Overall Prevalence of Extended-Spectrum β-Lactamases (ESβLs) (Table 2)

Within the scope of this study, 13 bacterial isolates exhibited characteristic synergy patterns resembling a champagne cork, indicative of ESβL production. This was observed among the 183 identified bacteria, constituting a prevalence of 7.1% (Table 2).

Table 2 ESβL Prevalence

Animals		**Cities**		**Bacteria**	
Types	**Number (%)**	**Tchibanga**	**Oyem**	***E. coli***	***Proteus mirabilis***
Goats	1 (7.7)	1 (7.7)	0 (00)	1 (7.7)	0 (00)
Pigs	12 (92.3)	0 (00)	12 (92.3)	11 (84.6)	1 (7.7)

4. Discussion

The primary objective of this study was to determine the different profiles and prevalence of antibiotic resistance among Gram-negative bacilli within livestock farms in Gabon. A total of 356 samples were collected, but only 183 samples gave positive culture. The majority of bacterial species in the digestive tract of the livestock were enterobacteria, including *Escherichia coli*, *Serratia*, *Enterobacter*, *Pantoea* spp, *Klebsiella*, *Citrobacter freundii*, *Proteus*, *Salmonella*, *Kluyvera* spp, among others. Additionally, non-enterobacterial Gram-negative bacilli such as *Stenotrophomonas maltophilia*, *Pseudomonas*, *Acinetobacter* spp, *Pasteurella pneumotropica*, and *Aeromonas hydrophila* were identified. These bacteria are found in both the digestive tracts of animals and humans, as well as in the environment, water, and soil. Most of them can be easily cultured in conventional culture media. In the environment and water, bacteria are likely contaminants associated with the food and living conditions of animals, especially during grazing [13]. In chickens, 15 species of Gram-negative bacilli were identified, with the most commonly found being *E. coli* and *P. oryzihabitans*. Only *E. coli* has been extensively studied as it is used as a sentinel bacterium [14]. For pigs, 22 species of bacteria were identified, with *E. coli* and *P. oryzihabitans* being the most frequent. However, antibiotic resistance has only been described in *Escherichia coli* and *Klebsiella* spp in Côte d'Ivoire [15] and South Africa. This resistance is the cause of serious and sometimes fatal infections in pigs [16].

In cattle samples, *E. coli* was the most frequently isolated species, followed by *P. oryzihabitans* and *Pantoa* spp. In previous studies in the field of livestock, only *E. coli*, *Pasteurella pneumotropica*, and *Salmonella choleraesuis* were most frequently isolated and identified, notably in Côte d'Ivoire [17]. Thus, livestock fauna possesses a diversity of Gram-negative bacilli of commensal and environmental origin in its gastrointestinal tract [18]. The diversity of Gram-negative bacilli in the gastrointestinal tract of livestock may be attributed to host specificity and dietary habits [19].

In this research, 20 antibiotics were used to study the antibiotic susceptibility of 183 bacteria. The susceptibility testing revealed high rates of acquired resistance among most identified bacteria (*E. coli*, *Salmonella choleraesuis*, *Proteus mirabilis*) in different farms. This situation is likely a consequence of the communal nature of strains [2] and the selective pressure from the widespread use of beta-lactams in veterinary medicine in Gabon. Continuous monitoring of resistance to Ceftriaxone and Cefotaxime is crucial due to their similar antimicrobial activity against Gram-negative bacteria.

The aminoglycosides (Gentamicin, Tobramycin, and Amikacin) maintained excellent activity, with high efficiency rates of 88.9%, 98.3%, and 97.8%, respectively, in pigs, chickens, cattle, and goats. These results align with Akoachere et al (2009). For quinolones (Ciprofloxacin, Nalidixic acid, Norfloxacin, and Ofloxacin), low resistance rates were observed in strains such as *E. coli*, *Proteus*, *S. choleraesuis*, *Stenotrophomonas maltophilia*, *Pantoea* spp, *Kluyvera* spp, and *Aeromonas hydrophila*, possibly due to the absence of first-level DNA gyrase (GyrA) mutations in *E. coli*, conferring resistance to nalidixic acid [20], and limited use of these antibiotics in livestock environments in Gabon [2]. However, resistance profiles varied across nine cities, likely reflecting local antibiotic consumption patterns [2, 21]. Thus, the city of Franceville has the highest antibiotic resistance rate at 43.9% among the 18 isolates. This high prevalence may be attributed to contamination during grazing, exposure to wastewater [13], antibiotic consumption in healthcare [22], or growth factors in animal feed [23]. Similar explanations may account for the observed antibiotic resistance prevalences in other cities in Gabon. However, the variation in antibiotic resistance rates among all cities is not statistically significant (p-Value = 0.16342). This similarity can be explained by the fact that in Gabon, the same antibiotics are used in both livestock and veterinary domains [2].

All isolated Gram-negative bacilli (GNB) exhibited multidrug-resistant (MDR) profiles. In chickens, *E. coli* and *Pseudomonas oryzihabitans* MDR showed a prevalence of 39.0%, similar to findings in Chad [24]. The prevalence of antibiotic resistance in pigs (38.8%) and chickens (39.0%) was comparable to studies in Osogbo (40.0% for pigs and 16.0% for chickens) [23], and slightly lower than in Ghana (58.2%) [25]. Resistances in cattle (27.0%) and goats (27.8%) were nearly similar to the Osogbo study (26.0%) [26] and different from results in Egypt (67%) [27].

Thirteen bacteria (7.1%) produced extended-spectrum beta-lactamases (ESβLs), comparable to Tunisia (11.2%) [23], Nigeria (16.7%) [23], and Egypt (56.0%) [27]. ESβLs confer high-level resistance to first and second-generation cephalosporins and varying reductions in the activity of third and fourth-generation cephalosporins and monobactams [28]. However, certain aminoglycosides (Gentamicin, Tobramycin) and second-class fluoroquinolones (Ofloxacin and Norfloxacin) remain highly effective against the present isolates due to their low resistance prevalences. The trend of high antibiotic resistance in the livestock sector has also been reported in other countries, such as Ghana, for isolates from normal intestinal flora in animals [25].

In the present study, the prevalence of multidrug-resistant bacteria (MDR) observed in pigs (38.8%), chickens (39.0%), cattle (27.0%), and goats (27.8%) was similar to that found in Osogbo, Nigeria, which was 40.0% (pigs), 16.0% (chickens), and 48.0% (cattle) [23]. These multidrug resistances indicate a high level of exposure to antibiotics either directly or indirectly (contaminated water, antibiotics-enriched feed, etc.) and the use of antibiotics in animals [2]. Although we did not address the issue of the consequences of antibiotic use on the health of consumers of these animals in this study, other studies report a high rate of antibiotic resistance in patients who have consumed meat from farm-raised chickens [29]. This underscores that bacterial antibiotic resistance can also be transmitted through the ingestion of meat. These different strains of MDR could be a significant cause of acute gastroenteritis in individuals of all age groups [30]. Hence, regular monitoring of antibiotic resistance in livestock is crucial

5. Conclusion

All identified isolates exhibited MDR, and a low prevalence of ESβLs (7.1%). As these MDR bacteria in farm animals are intended for human consumption, there is a significant risk of transmitting of these strains to consumers, posing a substantial threat to human health. This work serves as an alarm on food safety in Gabon.

Compliance with ethical standards

Acknowledgments

The collection of faecal samples was carried out in collaboration with Dr Désiré OTSAGHE EKORE, whose assistance is sincerely appreciated. The analysis of faecal samples was conducted using bacteriology consumables purchased with a grant from the « Agence Universitaire de la Francophonie » (AUF) at the Microbiology Laboratory of IRET. We express

our gratitude to AUF for this opportunity. Special thanks also go to the leadership of IRET for allowing the analysis of our samples to take place in the Microbiology laboratory.

Disclosure of conflict of interest

The authors declare that they have no competing interests.

Authors' contributions

Conceptualization, WBEC, PPMN and GRNA ; Methodology, PPMN, WBEC, YBP and RWL ; Software, PPMN ; Validation, PPMN ; Formal Analysis, PPMN, WBEC and RWL ; Investigation, PPMN ; Data curation, PPMN, WBEC and DOE ; Resources, DOE and PPMN ; Writing – Original Draft Preparation, PPMN ; Writing – Review & Editing, PPMN, GRNA and PMN ; Visualization, CRZK, JFM, EAL and PPMN ; Supervision, PPMN ; Project Administration, PPMN ; Funding Acquisition, PPMN.

Statement of ethical approval

This research was conducted in Gabon through a collaborative effort between IRET and the University of Science and Technology of Masuku (USTM) under Research Agreement No. 160/2020/MESRSTTENCFC/USTM/FS/Dn, dated August 7, 2020. This study was approved by the Gabonese Ministry of Agriculture, Livestock, Fisheries and Rural Development (Livestock Department authorization n0052/SG/DGE).

References

[1] Tonini, A., S.S. Matus, and S. Gomez y Paloma, A Bayesian Total Factor Productivity Analysis of Tropical Agricultural Systems in Central-Western Africa And South-East Asia. 2011.

[2] Ekore, D.O., et al., The antibiotics used in livestock and their impact on resistance in Enterococcus faecium and Enterococcus hirae on farms in Gabon. Antibiotics, 2022. 11(2): p. 224.

[3] Zasada, I., Multifunctional peri-urban agriculture—A review of societal demands and the provision of goods and services by farming. Land use policy, 2011. 28(4): p. 639-648.

[4] Soufi, L., et al., Prevalence and diversity of integrons and associated resistance genes in Escherichia coli isolates from poultry meat in Tunisia. Foodborne Pathogens and Disease, 2009. 6(9): p. 1067-1073.

[5] Barlow, R.S., J.M. Pemberton, P.M. Desmarchelier, and K.S. Gobius, Isolation and characterization of integron-containing bacteria without antibiotic selection. Antimicrobial agents and chemotherapy, 2004. 48(3): p. 838-842.

[6] Hazards, E.P.o.B., et al., Role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain. Efsa Journal, 2021. 19(6): p. e06651.

[7] Aarestrup, F.M. and H. Hasman, Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection. Veterinary microbiology, 2004. 100(1-2): p. 83-89.

[8] Bonnet, R., Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrobial agents and chemotherapy, 2004. 48(1): p. 1-14.

[9] Chishimba, K., et al., Detection of extended-spectrum beta-lactamase-producing Escherichia coli in market-ready chickens in Zambia. International journal of microbiology, 2016. 2016.

[10] Mbehang Nguema, et al., Characterization of ESBL-Producing Enterobacteria from Fruit Bats in an Unprotected Area of Makokou, Gabon. Microorganisms, 2020. 8(1): p. 138.

[11] Wasyl, D., A. Hoszowski, M. Zając, and M. Skarżyńska, Simple and efficient screening method for the detection of cephalosporin resistant. Bull Vet Inst Pulawy, 2010. 54: p. 147-51.

[12] Bauer, A., W. Kirby, J.C. Sherris, and M. Turck, Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology, 1966. 45(4): p. 493.

[13] Bueno, I., et al., Identifying the spatiotemporal vulnerability of soils to antimicrobial contamination through land application of animal manure in Minnesota, United States. Science of the Total Environment, 2022. 832: p. 155050.

[14] Mbehang Nguema, P.P., S. Tsuchida, and K. Ushida, Bacteria culturing and isolation under field conditions of Moukalaba-Doudou National Park, Gabon, and preliminary survey on bacteria carrying antibiotic resistance genes. Tropics, 2015a. 23(4): p. 165-174.

[15] Koné, A., et al., Antibiotic Resistance Profile of Escherichia coli and Klebsiella spp Isolated from Post-weaned Piglet Faeces under Penicillin-streptomycin Combination Therapy (Penstrep®) in Abidjan, Côte d'Ivoire.

[16] Parisi, A., et al., MRSA in swine, farmers and abattoir workers in Southern Italy. Food microbiology, 2019. 82: p. 287-293.

[17] Kouadio, I., et al., Detection of Resistance Integron in Escherichia coli of Porcine Origin Producing Extended-spectrum Beta-lactamase in Abidjan, Côte d'Ivoire. Journal of Advances in Microbiology, 2018. 8(3): p. 1-7.

[18] Seiffert, S.N., M. Hilty, V. Perreten, and A. Endimiani, Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: an emerging problem for human health? Drug Resistance Updates, 2013. 16(1-2): p. 22-45.

[19] Senghor, B., C. Sokhna, R. Ruimy, and J.-C. Lagier, Gut microbiota diversity according to dietary habits and geographical provenance. Human Microbiome Journal, 2018. 7: p. 1-9.

[20] Jaktaji, R.P. and E. Mohiti, Study of mutations in the DNA gyrase gyrA gene of Escherichia coli. Iranian journal of pharmaceutical research: IJPR, 2010. 9(1): p. 43.

[21] Mbehang Nguema, P.P., et al., High level of intrinsic phenotypic antimicrobial resistance in enterobacteria from terrestrial wildlife in Gabonese national parks. PLoS One, 2021. 16(10): p. e0257994.

[22] Tian, M., et al., Pollution by antibiotics and antimicrobial resistance in livestock and poultry manure in China, and countermeasures. Antibiotics, 2021. 10(5): p. 539.

[23] Adesiji, Y.O., O.T. Alli, M.A. Adekanle, and J.B. Jolayemi, Prevalence of Arcobacter, Escherichia coli, Staphylococcus aureus and Salmonella species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. Sierra Leone Journal of Biomedical Research, 2011. 3(1): p. 8-12.

[24] Bodering, A., et al., Characteristics of poultry farms and assessment of their level of contamination by Salmonella spp. and Escherichia coli in the towns of N'Djamena and Doba in Chad. Rev. Sci. Tech. Off. Int. Epiz, 2018. 37(3): p. 2.

[25] Osei Sekyere, J., Antibiotic types and handling practices in disease management among pig farms in Ashanti Region, Ghana. Journal of veterinary medicine, 2014. 2014.

[26] Sarba, E.J., et al., Identification and antimicrobial susceptibility profile of Escherichia coli isolated from backyard chicken in and around ambo, Central Ethiopia. BMC veterinary research, 2019. 15: p. 1-8.

[27] Aly, M.E., T.M. Essam, and M.A. Amin, Antibiotic resistance profile of E. coli strains isolated from clinical specimens and food samples in Egypt. International Journal of Microbiological Research (IJMR), 2012. 3(3): p. 176-182.

[28] Houchi, S., Characterization of ß-lactamase genes in clinical isolates and determination of the inhibitory effects of Algerian seaweeds extracts on recombinant ß-lactamases GES-22 and OXA-1. 2019.

[29] Guetiya Wadoum, R., et al., Abusive use of antibiotics in poultry farming in Cameroon and the public health implications. British poultry science, 2016. 57(4): p. 483-493.

[30] Eltai, N.O., et al., Antibiotic resistance and virulence patterns of pathogenic Escherichia coli strains associated with acute gastroenteritis among children in Qatar. BMC microbiology, 2020. 20: p. 1-12.