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Phenotypic Characterization of Antibiotic Resistance in *Escherichia coli* Strains Isolated from Bovine Faeces in the District of Abidjan

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Abstract

Meat of bovine origin is one of the main vectors for the transmission of *Escherichia coli* to human consumers. This pathogen can cause serious human illness. **Objective:** The objective of this study was to characterize the resistance phenotype of *Escherichia coli* isolates isolated from bovine dung in the Abidjan district. **Methods**: A total of 336 cattle dung samples were analyzed and 23.80% (80/336) were positive for *Escherichia coli*. **Results**: The most common phenotypic resistances detected were 42.9% for nalidixic acid, 33.3% for amoxicillin, 30% for amoxicillin/clavulanic acid, 25% for enrofloxacin, 20% respectively for cefepime and trimethoprim-sulfamethoxazole. However, no resistance was observed to ticarcillin, ceftazidime, gentamicin and amikacin. In total, 70% (56/80) of isolates were resistant to at least three (3) antibiotic families, and 20% (16/80) were resistant to at least four (4) families. These results suggest that *Escherichia coli* isolates from bovine faeces are significantly diverse in terms of antibiotic resistance. **Conclusion**: the gastrointestinal microflora of cattle could be an important reservoir of various determinants of antimicrobial resistance, which is potentially dangerous for public health.

Keywords

Escherichia coli, Antibiotics, Resistance, Bovine, Côte d'Ivoire

1. Introduction

Ruminant farming in Côte d'Ivoire is underdeveloped and the country remains dependent on neighbouring countries such as Mali and Burkina Faso for its supplies. In large urban cities, beef is the source of animal protein [1] and therefore occupies an important place in the daily diet of the population. This situation has led to the appearance of local livestock farming, also known as urban and peri-urban livestock farming, which supplies the livestock market and slaughterhouses. In the district of Abidjan, the breeding of these ruminants contributes to the fight against food insecurity, by supplying slaughterhouses and creating jobs [1]. In addition, this type of urban livestock farming represents an important source of income for the population and contributes greatly to the fight against poverty [2], [3]. The excrement of these animals is generally used as fertilizer for vegetable crops in cities [1] making the animal sector a risk to public health.

The bacterium *Escherichia coli* is a bacterial species mainly present in the digestive tract of humans and animals in a commensal manner, but it can also be pathogenic.

The *Enterobacteriaceae* family is commonly used as an indicator of fecal contamination during microbiological analyses of food. It includes important zoonotic bacteria such as *Salmonella* spp., *Yersinia* spp. and *Escherichia coli*, *Klebsiella* spp*. Enterobacteriaceae* are important causes of serious infections, and many of their most important members are becoming increasingly resistant to currently available antimicrobials [4].

In fact, cattle can also harbour antibiotic-resistant *Escherichia coli* strains. These strains of pathogenic *Escherichia coli* and/or antibiotic-resistant foods can be transmitted to humans either through direct contact with cattle or indirectly through the ingestion of raw or undercooked bovine feed, but also through the environment. Hence the importance of considering, in a One Health context, that antibiotic resistance in environments impacted by cattle represents a major health issue [1]. The indiscriminate use of antibiotics in animals and especially in cattle to promote growth and prevent disease is directly linked to the incidence of resistant bacteria in their resident microbiota [5] [6] that could potentially contaminate the carcass during the slaughter process or subsequent handling and be transmitted to consumers and distributed in the environment [7] [8].

This antibiotic resistance is currently one of the greatest threats to health, food security and development in the world. Resistance to ampicillin (92.5%), tetracycline (76.6%) and trimethoprim/sulfamethoxazole (70.1%), 51.9% to sulfamethoprim, 26.9% to neomycin and 9.6% to enrofloxacin has been reported [9]. The epidemiological evolution of resistant pathogenic bacteria around the world is a warning of a potential danger for food safety.

Unfortunately, in Côte d'Ivoire, apart from the work of Yao [1], there are very few data on cattle farming in the development of antibiotic resistance and its possible transfer to humans.

In this study, the objective was to determine the level of resistance of *Escherichia coli* strains isolated from bovine dung on a cattle farm in the district of Abidjan.

2. Material and Methods

2.1. Material

Study Area

Samples were collected in four communes in the district of Abidjan (Abobo, Adjamé, Yopougon and Bingerville) and in the commune of Jacqueville located in the district of Lagunes. The district of Abidjan located at 5˚20'00" N, 4˚01'00" W is composed of 13 communes that extend over 2119 km^2; it is the most populous city in French-speaking West Africa with more than 6.321 million inhabitants [10].

The commune of Jacqueville, whose geographical coordinates are 5˚12'0" N and 4˚30'0" W, is a city located in the south of Côte d'Ivoire, about 60 km from the Abidjan agglomeration, in the Grands-Ponts region. Its population is estimated at 49,694 [10]. These municipalities were selected for the presence of many types of livestock (poultry, cattle, rabbits) and the agreement of the owners of the farms to participate in the study. Parks are individual but usually community parks with cattle owned by several owners.

The study area has two rainy seasons and two dry seasons [11]. It is characterized by abundant rainfall (1500 mm of water/year) and an average temperature of 27˚C and the average annual humidity level is more than 80%.

Collection sites

Collection sites were recruited on the basis of the presence of groups of animals in cattle pens belonging to either a single individual or several owners and the agreement of the breeders to participate in the study. In each municipality visited, it was necessary to explain the objectives of this study in order to obtain the agreement of the park owner(s) to participate in the study. Then, each park representing an epidemiological unit was selected on the basis of an estimated population of 50 to 100 head of adult beef cattle intended for sale for the consumption of local populations.

Type and period of study

This is a descriptive cross-sectional study that took place over a six-month period from January to June 2024. Microbiological analyses were carried out at the National Reference Center for Antibiotics at the Institut Pasteur de Côte d'Ivoire, Adiopodoumé site. The antibiotic susceptibility test was carried out at the Antibiotics, Natural Substances and Microorganism Surveillance to Anti-Infectives Unit of the Institut Pasteur de Côte d'Ivoire, Cocody site.

Study population

The study population consisted of beef cattle herds intended for human consumption, consisting mainly of adult cattle of the Zebu breed.

Inclusion criteria

The study involved adult cattle between 2 and 4 years of age, apparently healthy, *i.e.* without observable pathological signs and awaiting slaughter for sale for human consumption.

Young animals less than two years of age, sick animals and those undergoing treatment were not included in this study.

2.2. Methods

Sampling

In each park visited, approximately 5 to 10 apparently healthy cattle were selected based on the estimated number of cattle on a random basis. Using long, sterile gloves, a quantity of about 100 grams of faeces was collected directly from the rectum of the animals and put in sterile collection jars. The samples collected were immediately placed in a cooler containing cold accumulators and then transported to the laboratory of the National Reference Center for Antibiotic Resistance (CNR/RAM) of the Institut Pasteur de Côte d'Ivoire, Adiopodoumé site.

Collection form

A collection sheet was used to collect field data on the parks and animals. This information concerned: general data on the park (collection location, type of farming, information on the owner(s), animal data (animal species, breed, estimated number of animals) and treatments administered (current treatments, commonly used medications).

Surveys

The surveys were carried out in parallel with the collection of samples. A pre-established questionnaire was administered to individual breeders on the geographical location of the park or farm, the owners, the different breeds and numbers of animals, the diseases commonly observed, the treatments administered with particular emphasis on the antibiotic molecules used during the last 10 days prior to the visit. Ongoing treatment during the visit was a criterion for excluding animals from the study.

Sample transport

The samples were transported in a cooler with cold packs to the laboratory of the National Reference Center for Antimicrobial Resistance (CNR-RAM) at the Institut Pasteur de Côte d'Ivoire (Adiopodoumé site).

Isolation and identification

Approximately 25 g of fecal sample was dissolved in 225 mL of buffered peptone water (BPW) and incubated for 24 hours at 37˚C. The next day, the broth was isolated using the streak method on an Eosin Methylene Blue (EMB) agar and pure cultures of the isolates were obtained after 24 hours of incubation at 37˚C. All colonies with a suspicious typical metallic reflection on EMB were subjected to morphological and biochemical tests using conventional bacteriological methods.

Antibiotic Susceptibility Testing

The antibiotic susceptibility test was carried out in accordance with the EUCAST-CASFM version 2023 standard.

➢ **Preparation of the inoculum**

From a 24-hour pure bacterial culture on ordinary agar, 2 to 3 colonies were taken from the loop and emulsified in 2 ml of saline water (0.85% NaCl, BioMérieux®, France) to obtain a turbidity of 0.5 on the MacFarland scale, which corresponds to a bacterial concentration of 10^6 CFU/ml.

➢ **Muller-Hinton agar inoculation and application of discs**

Kirby Bauer's modified WHO-recommended method based on diffusion from antibiotic-impregnated discs on Muller-Hinton agar [12] was used to perform the antibiotic susceptibility test. Help sterile swab sticks soaked in the bacterial suspension, each isolate was inoculated with tight streaks over the entire surface of the Müller-Hinton agar. The inoculum should be evenly distributed over the entire surface of the agar, taking care not to leave any space between the streaks. A panel of twelve antibiotic discs (Bio Mérieux France) including: amoxicillin (20 µg), amoxicillin-clavulanic acid (20/10 µg), ticarcillin (75 µg), cefoxitin (30 µg), ceftazidime (30 µg), cefepime (30 µg), gentamicin (10 µg), amikacin (30 µg) trimethoprim-sulfamethoxazole (25 µg), tetracycline (30 µg), nalidixic acid (30 µg), enrofloxacin (5 µg) were used in the study. For sensitivity testing, a Reference strain *Escherichia coli ATCC*25922 was used as an internal quality control for antibiotic susceptibility testing.

After incubation, the reading and interpretation of the results was done using BioRad's ADAGIO® automatic reader (BIORAD France).

Detection of the ESBL phenotype according to the double synergy test

All strains of *Escherichia coli* isolated cells were detected for the production of extended-spectrum beta-lactamase by the double diffusion or double synergy assay. It is conducted under the standard conditions of the antibiogram. Ceftazidime and cefepime discs were placed around an amoxicillin/clavulanic acid disc on Müller-Hinton agar (approximately 15 mm) and then incubated at 37˚C for 24 hours. The appearance of an image in the shape of a champagne cork reflects the production of an extended-spectrum beta-lactamase by the strain of *Escherichia coli* tested [13].

Statistical analyses

All the qualitative variables were described by frequencies and percentages using the "Excel" tool.

3. Results

During the study period, 34 cattle yards were visited, three (3) of which were excluded due to ongoing treatment for pathological problems.

3.1. Sampling

During the study period, a total of 336 samples were collected in the various parks selected. The distribution of the samples according to the municipalities is summarized in Figure 1.

3.2. Prevalence of Detected *E coli* Strains

The analysis of the results revealed the presence of *Escherichia coli* strains in the faeces of cattle in all the parks recruited. Thus, out of a total of 336 cattle samples taken, 80 strains of *Escherichia coli* were identified (Table 1), *i.e.* a prevalence of 23.80%.

3.3. Antibiotic Susceptibility of Strains

The antibiotic resistance study showed that *Escherichia coli* strains had high rates of resistance to commonly used antibiotics such as nalidixic acid (42.9%), amoxicillin (33.3%), amoxicillin/clavulanic acid (30%), enrofloxacin (25%), cefepime (20%) and trimethoprim-sulfamethoxazole (20%). These strains showed no resistance to ticarcillin, ceftazidime, gentamicin and amikacin (see **Figure 2** below).

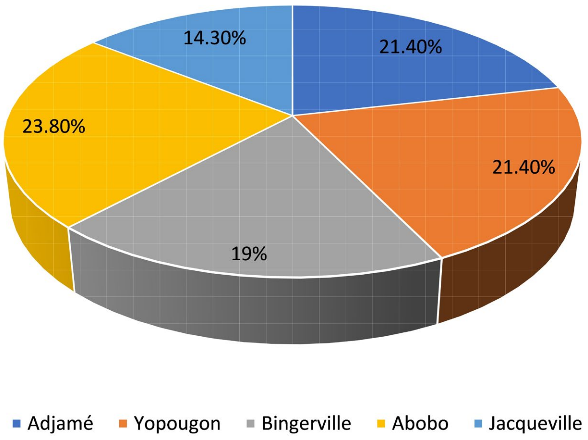
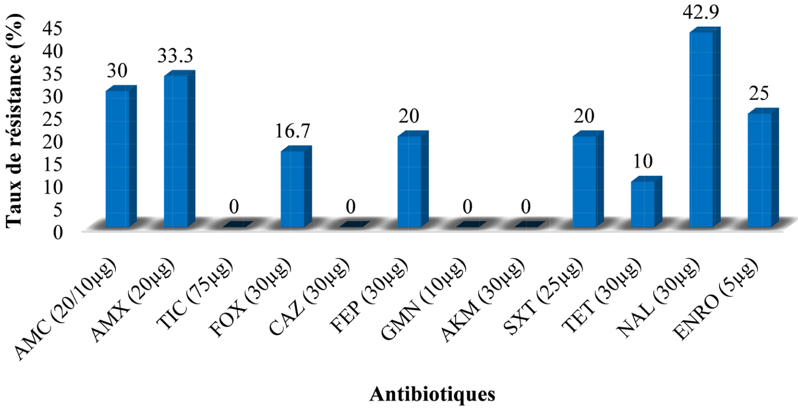


Figure 1. Distribution of samples collected according to municipalities.

Table 1. Distribution of *Escherichia coli strains* isolated according to the municipalities.

COMMON	Number (n)	Percentages (%)
Adjamé	16	20
Yopougon	24	30
Bingerville	8	10
Abobo	16	20
Jacqueville	16	20
Total	80	100



AMC: amoxicillin/clavulanic acid, AMX: amoxicillin, TIC: ticarcillin, FOX: cefoxitin, CAZ: ceftazidime, FEP: cefepime, GMN: gentamycin, AKM: amikacin, SXT: trimethoprim/sulfamethoxazole, TET: tetracycline, NAL: nalidixic acid and ENRO: enrofloxacin.

Figure 2. Antibiotic resistance rates of *Escherichia coli* strains isolated from bovine faeces.

3.4. Extended Spectrum Beta-Lactamase (*ESBL*)-Producing Strains of *Escherichia coli*

Of the 80 strains of *Escherichia coli* isolated from bovine faeces, no strain was categorized as producing Extended Spectrum Beta-lactamase (*ESBL*) based on the detection of double synergy. However, the results reveal that the bacteria expressed resistance to one or more antibiotics (Table 2).

Table 2. Multidrug resistance in *Escherichia coli* strains isolated from bovine faeces.

Multi-resistance of isolated bacteria	Number of Bacterial Strains (n)	Percentage (%)
AMX	8	10
AMC/NAL	8	10
AMC/SXT/TET	8	10
AMC/FEP/NAL/ENRO AMX/SXT/NAL/ENRO AMC/SXT/TET/NAL	24	30
AMC/SXT/TET/NAL/ENRO	8	10
AMC/FEP/SXT/TET/NAL/ENRO AMC/FOX/FEP/SXT/NAL/ENRO	16	20
AMX/AMC/FOX/FEP/SXT/NAL/ENRO	8	10
TOTAL	80	100

AMX = amoxicillin; AMC = amoxicillin + clavulanic acid; FEP = cefepime; SXT = trimethoprim + sulfamethoxazole; TET = tetracycline; FOX = cefoxitin; NAL = nalidixic acid; ENRO = enrofloxacin.

3.5. Multi-Resistance of *Escherichia coli* Strains to the Antibiotics Tested

The results in Table 2 show that 8 strains of *E. coli* (10%) showed resistance to only one antibiotic (amoxicillin), while the majority of strains (90%) developed resistance to at least 2 antibiotics. In addition, this multi-resistance has affected several families of antibiotics (penicillins, cephalosporins, quinolones and fluoroquinolones, tetracyclines, other antibiotics such as the combination of trimethoprim + sulfamethoxazole).

In Table 3, the results obtained showed that 70% of the strains showed resistance to at least three (3) families of antibiotics and 20% to at least four (4) families of antibiotics. The families of antibiotics concerned are beta-lactams, sulfonamides and cyclins.

Table 3. Distribution of *Escherichia coli* strains resistant to several families of antibiotics.

Number of inactive antibiotic families	**Families**	Molecules	Number (%) **(N = 80)**
One family	**Beta-Lactam**	Amoxicillin	**8 (10)**

Continued

Two families	**Beta-lactam**	Amoxicillin/Clavulanic Acid Cefepime	**40 (50)**
	Quinolones/Fluoroquinolones	Nalidixic acid Enrofloxacin	**48 (60)**
Three families	**Beta-lactams-Sulfonamides-Cyclins**	Amoxicillin/Clavulanic Acid Sulfamethoxazole/Trimethoprim Tetracycline	**48 (60)**
	Beta-Lactams-Sulfonamides-Quinolones/ Fluoroquinolones	Amoxicillin Amoxicillin/Clavulanic Acid Cefoxitin Cefepime Sulfamethoxazole/Trimethoprim Nalidixic acid Enrofloxacin	**8 (10)**
Four families	**Beta-Lactams-Sulfonamides-Cyclins-Quinolones/ Fluoroquinolones**	Amoxicillin/Clavulanic Acid Cefepime Sulfamethoxazole/Trimethoprim Tetracycline Nalidixic acid Enrofloxacin	**16 (20)**

4. Discussion

The strains of *Escherichia coli* isolated from bovine faeces have been isolated in 23.80% of the levies. A lower prevalence (5.38%) was reported respectively by [14] and by Kayantao and al. [15] in Mali (8.28%). Nevertheless Oubayyou *et al.* reported a similar value (21.55%) in Niger [16]. However, all these results are lower than the one reported by Ouédraogo *et al.* in 2010 in Ouagadougou (29.2%) [17] and by Horo *et al.* in 2004 in Abidjan (31.92%) [18]. According to studies, bacterium *Escherichia coli* is a commensal of the digestive tract of humans and many animals, it represents the dominant species of the aerobic bacterial forest in the natural digestive flora of the intestine [19] [20], which would explain its high prevalence in animals.

Concerning beta-lactams, our study shows a resistance of 33.3% to amoxicillin, 30% for the combination amoxicillin/clavulanic acid, 16.70% to cefoxitin and 20% to cefepime, while ticarcillin and ceftazidime were found to be sensitive for all strains tested Probably because these molecules are not commonly used in veterinary medicine. Gay's work on *E coli* from diarrhoea and mastitis in cows with regard to amoxicillin give levels of 85% and 30% respectively; The author explains that the differences can be observed depending on the bovine pathologies considered [21]. As for amoxicillin-clavulanate, the rate of 30% is well above the Roussel results for strains isolated from gastrointestinal samples (3.2%) [22]. Most commonly, clavulanic acid is not sufficient to restore sensitivity to amoxicillin in penicillinase-producing Enterobacteriaceae [23]. Indeed, this resistance to amoxicillin + clavulanic acid allows us to hypothesize a decrease in the activity of beta-lactamase inhibitors probably due either to an overproduction of penicillinases or to the inactivation of the inhibitor itself [24] [25]. However, some strains of *Escherichia coli* were suspected of producing cephalosporinase with cefoxitin (16.70%) and cefepime (20%), contrary to Perrin's results in 2021 which indicate a level of 0.3 to 0.6 in beef [26]. According to the same author, the percentage of *Escherichia coli* producer of *β*-Extended-spectrum lactamases (ESBLs) or cephalosporinases (ESBLs/AmpCs) are low, especially in foodstuffs. Some enzymes that hydrolyze broad-spectrum cephalosporins are thought to have initially derived from plasmid narrow-spectrum penicillinases and were mainly found in hospital strains [25] [27].

In the case of quinolones and fluoroquinolones, resistance rates vary according to the pathologies [21]. The Results in this study show that strains of *Escherichia coli* Isolated were resistant to 42.9% to nalidixic acid and 25% to enrofloxacin, A similar study shows resistance to nalidixic acid 17.9%, norfloxacin 11.3% and ciprofloxacin 10.6% for strains isolated from gastrointestinal specimens [22].

All strains in this study were sensitive to aminoglycosides, as indicated by the work in 2025 [28], but a study carried out in Morocco in 2006 showed resistance of 16.7% for gentamicin and 12.6% for amikacin [29].

The percentages of resistance to tetracycline and trimethoprim-sulfamethoxazole were 10% and 20%, respectively, while Gay in 2008 found higher percentages in *Escherichia coli* for the combination of trimethoprim-sulfonamides (64%) and tetracycline (83%) (Gay *et al.*, 2008).

The results of this study show multidrug resistance in *Escherichia coli* isolated from bovine faeces to two families, three families, or even four families of antibiotics with levels ranging from 50 to 60% (see **Table 3**). This rate is well above the results of Résapath in 2020 [30] showing higher percentages of multidrug-resistant strains (MDR) among isolates from cattle (15%) compared to rates found in other animals (swine, poultry, companion animals). Resistance patterns highlight disparities between animal species and disease context within the same species, with 18% of bovine isolates from *Escherichia coli* are MDRs among strains isolated in digestive pathology, compared to only 3% for isolated strains of mastitis [31]. According to this author, resistance carried by plasmids can be transferred from one strain to another or from one bacterial species to another, thus accelerating their spread. The accumulation of resistance mechanisms in a bacterium can lead to therapeutic dead ends.

In general, the antibiotic resistance of *Escherichia coli* strains isolated from bovine faeces was high for nalidixic acid, an antibiotic molecule against which most bacterial strains showed a higher resistance (42.9%). Resistance of less than 20% on the strains tested was found, to tetracycline, cefepime, cefoxitin, tetracycline and trimethoprim-sulfamethoxazole. The low rate of resistance observed in our study should not mask the current emergence of antibiotic resistance in Côte d'Ivoire [1] [32] [33]. Several studies showed intestinal microbiota to be a reservoir of antimicrobial resistant bacteria [34] [35].

Studies show that a bacterial strain can be resistant to several antibiotic families simultaneously (multidrug-resistant) and that the use of one antibiotic will co-select resistance to other antibiotic families, thus helping to enrich the bacterial population with multidrug-resistant strains [36].

The prevalence of *Escherichia coli* detected could highlight the need for further surveillance to identify critical points of contamination. In addition, the resistance rates of strains resistant to several antibiotics indicate that cattle may have been exposed to the inappropriate or excessive use of antibiotics and are an important reservoir of antibiotic-resistant bacteria acting as a means of spread.

5. Conclusion

This study revealed the presence of *Escherichia coli* in cattle farms intended for human consumption. A high prevalence of BMR has been observed and resistance has affected the families of antibiotics most often used in human therapeutics, such as beta-lactams, fluoroquinolones and in chemoprophylaxis, particularly in people living with HIV such as cotrimoxazole.

Nowadays, the gut microbiota is recognized as the place where many antibiotic-resistant bacteria emerge, multiply and spread within a population, making it a key player in the proliferation of antibiotic-resistant infections. Contamination of the environment by these germs can lead to the transmission of infectious diseases but also of bacteria carrying genes of resistance to humans from infected faeces present in the environment, in meat and milk, but also contamination by direct contact between humans and animals and also between animals.

6. Limitations of the Study

This preliminary study can be improved by a larger sample size. However, these results clearly show the public health problem that resistant bacteria represent in animal health.

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Conflicts of Interest

The authors state that there is no conflict of interest.

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