

Comparative analysis of antimicrobial resistance profiles of *Campylobacter* isolated from broiler chickens, cattle and sheep in Algeria

Análisis comparativo de los perfiles de resistencia a los antimicrobianos de cepas de *Campylobacter* aisladas de pollos de engorde, ganado vacuno y ovino en Argelia

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ABSTRACT

Among the pathogens associated with antimicrobial resistance, *Campylobacter jejuni* and *Campylobacter coli* are the major etiological agents of gastrointestinal infections worldwide. This study aimed to explore the frequency of antibiotic-resistant *Campylobacter* isolates among broiler chickens, cattle and sheep in Algeria, conducting a comparative analysis according to animal sources. A total of 138 isolates (96 of *C. jejuni* and 42 of *C. coli*) collected from meat samples of broiler chickens, cattle and sheep. The antimicrobial testing was conducted using disk diffusion methods. The findings revealed a high resistance in broiler chickens compared to other animal origins. Specifically, *Campylobacter coli* isolates from chickens demonstrated a complete resistance (100 %) to quinolones, fluoroquinolones, macrolides, and tetracycline, followed by *Campylobacter jejuni*, which showed resistance to quinolones, fluoroquinolones, and metronidazole (92 %), macrolides (75 %), and beta-lactams (69 %). Bovine *C. coli* isolates exhibited total resistance to quinolones, along with an increasing resistance to cephalosporins and macrolides. Similarly, ovine *C. coli* isolates demonstrated significant resistance to both beta-lactams and nitroimidazoles (70 %). Statistical analysis through variance testing, revealed high antibiotic resistance profiles in chickens compared to bovine and ovine isolates ($P < 0.05$). Bovine and ovine isolates exhibited elevated rate for quinolones and their resistance were less extensive than observed in broiler chickens. The results underscore the importance of considering the origin of isolates when evaluating resistance profiles in order to limit the spread of antimicrobial resistance under the one health approach.

Key words: Antimicrobial resistance; *Campylobacter*; broiler chickens; cattle; sheep.

RESUMEN

Campylobacter son los principales agentes etiológicos de las infecciones gastrointestinales. El objetivo de este estudio fue analizar la frecuencia de cepas de *Campylobacter* resistentes a los antibióticos en pollos de engorde, ganado vacuno y ovino de Argelia, mediante un análisis comparativo por origen animal. Se recogieron un total de 138 cepas (96 de *Campylobacter jejuni* y 42 de *Campylobacter coli*). Las pruebas de sensibilidad a los antimicrobianos se realizaron mediante métodos de difusión en disco. Los resultados revelaron una mayor resistencia en los pollos de engorde que en los otros orígenes, con una mayor tasa de *C. coli* que de *C. jejuni* con respecto a las quinolonas, las fluoroquinolonas, los macrólidos, la tetraciclina (100 %) y los betalactámicos (72-77 %) en las cepas de *C. coli*, seguidas de un alto nivel de resistencia en *C. jejuni* frente a las quinolonas, las fluoroquinolonas y el metronidazol (92 %), macrólidos (75 %) y los betalactámicos (69 %). En el ganado vacuno y ovino también se obtuvieron tasas elevadas: las cepas de *C. coli* bovina mostraron una resistencia total a las quinolonas, un nivel creciente de resistencia a las cefalosporinas y los macrólidos (79 % y 59 %, respectivamente), y la *C. coli* ovina presentó una tasa elevada de resistencia a los betalactámicos y los nitroimidazoles (70 %). En el análisis de varianza, se observaron perfiles de alta resistencia en los pollos de engorde a la mayoría de los antibióticos, en comparación con las cepas bovinas y ovinas, con diferencias estadísticamente significativas ($P < 0,05$). Las cepas bovinas y ovinas mostraron tasas elevadas de resistencia a las quinolonas, aunque esta resistencia fue menos extensa que la observada en los pollos de engorde. Con el fin de limitar la propagación de la resistencia a los antimicrobianos, es necesario adoptar un enfoque «Una sola salud».

Palabras clave: Resistencia a los antimicrobianos; *Campylobacter*; pollos de engorde; ganado bovino; ovejas.

INTRODUCTION

Among global health problems, antimicrobial resistance (AMR) is a prominent example of the One Health approach [1, 2]. In food-producing animals, antimicrobial resistance can be transmitted to humans through foodborne pathways, as observed with bacteria such as *Campylobacter* spp., *Salmonella* spp., and *Escherichia coli*, as well as through environmental contamination and direct contact with animals [3, 4].

Of the pathogens associated with AMR, *Campylobacter* species, especially *C. jejuni* and *C. coli*, are leading contributors to gastrointestinal infections [5], and the most frequently reported as a zoonotic etiological agents affecting both humans and animals worldwide [6, 7].

Campylobacteriosis is primarily associated with the consumption and cross-contamination of food occurring in the preparation of slaughtered animal carcasses [8]. Key risk factors for infection include the consumption of undercooked or uncleaned meat, improper handling of raw foods, cross-contamination between raw and cooked foods, and direct contact with contaminated animals or animal carcasses [9]. Poultry and poultry products have a long been considered as a major source and reservoir of human infections, accounting for 50-70 % of all reported cases of Campylobacteriosis followed by ruminants (beef, dairy cattle, and their manure) [6, 10].

Although *Campylobacter* infections in humans are sporadic and often self-limiting, antimicrobial therapy is indicated in severe and prolonged cases [11]. The campylobacteriosis treatment is mainly based on fluoroquinolones (ciprofloxacin) due to their broad-spectrum activity, and effectiveness against both Gram-negative and Gram positive bacteria [6, 12].

In food-producing animals, fluoroquinolones are frequently used to treat infections and in same case, have been indiscriminately employed as feed additive and growth promoters [13, 14]. The rise in resistance to fluoroquinolones has necessitated the introduction of a new class of antibiotics. Macrolides are currently first-line treatment for campylobacteriosis [15], with erythromycin being the most widely used agent, demonstrating satisfactory therapeutic results.

On the other hand, erythromycin resistance levels have been increasing in recent years [16, 17], requiring urgent active surveillance [13]. In such situations, alternative antibiotics such as tetracycline and gentamicin, or amphenicols such as chloramphenicol may be also used [18].

The widespread exposure of *Campylobacter* to antibiotics, used in both human and Veterinary Medicine, has created selective pressure that has driven the development and rising prevalence of antibiotic resistant associated with outbreaks throughout the world [9, 19]. This is largely due to the significant prevalence of these bacteria in the environment and their ability to colonise the gastrointestinal tracts of various animals [20].

Livestock operations particularly large-scale, consistent, and heavily packed may amplify threat of zoonotic diseases in surroundings environments [21]. The resistance cannot be attributed solely to antibiotic use, as interspecies transmission and subsequent clonal expansion also contribute to the spread

of antibiotic-resistant *Campylobacter* [19]. However, resistance can be developed through spontaneous mutations and the acquisition of resistance determinants via natural transformation, transduction, or conjugation [22], according to their different mechanisms of evasion against each antibiotic.

Given these circumstances, this study aimed to explore the prevalence of antibiotic-resistant *Campylobacter jejuni* and *Campylobacter coli* isolates among broiler chickens (*Gallus gallus domesticus*), cattle (*Bos taurus*) and sheep (*Ovis aries*) in Algeria. Additionally, it sought to conduct a comparative analysis of the monitoring results according to isolates animal origins in order to assess the impact of animal origins on resistant patterns highlighting potential reservoirs of resistant isolates. Notably, there is limited comparative data on the antibiotic resistance profiles of *Campylobacter jejuni* and *Campylobacter coli* isolated from broiler chickens compared to those from other animal species, particularly in developing countries.

MATERIALS AND METHODS

Study design and *Campylobacter* isolates

The study was conducted during 2022 to 2024. A total of 138 *Campylobacter* isolates were collected from meat samples of broiler chickens, cattle and sheep during the slaughtering process at slaughterhouses located in three eastern Algerian provinces (Batna, Bordj Bou Arreridj and Setif) including 96 of *Campylobacter jejuni* and 42 of *Campylobacter coli* (TABLE I).

TABLE I
Campylobacter isolates distribution and origins of meat samples

Animal origin	Isolates number	<i>C. jejuni</i>	<i>C. coli</i>
Broiler Chickens	83	65	18
Sheep	30	16	14
Cattle	25	15	10
Total	138	96	42

Thus, 83 isolates (65 *C. jejuni* and 18 *C. coli*) were collected from broiler chickens, with 40 from Batna province, 28 from Borj Bou Arreridj and 15 from Setif. Additionally, 29 meat samples from cattle (15 *C. jejuni* and 14 *C. coli*) and 25 from sheep (15 *C. jejuni* and 10 *C. coli*) were collected, primarily from Batna and other eastern provinces.

The isolation and identification of *Campylobacter* spp. isolates were carried out according to the ISO 10272-2: 2023 guidelines [23], allowing the identification of *C. jejuni* and *C. coli* isolates which were subsequently subjected for antimicrobial susceptibility testing.

Antimicrobial susceptibility tests of *C. jejuni* and *C. coli*

Antimicrobial susceptibility testing of *Campylobacter jejuni* (n = 96) and *Campylobacter coli* (n = 42) isolates was performed using the disk diffusion method in accordance with the guidelines of CASFM clinical breakpoints [19]. Bacterial suspensions were

prepared in sterile saline and adjusted to a turbidity equivalent to a 0.5 McFarland standard. The suspensions were then inoculated onto Mueller–Hinton agar (Oxoid Ltd., France) supplemented with 5 % lysed horse blood. Control strains (*C. jejuni* ATCC 33560 and *C. coli* ATCC 33559) were also used. The plates were incubated under microaerophilic conditions at 41.5 °C for 24–48 h.

A total of 14 antimicrobial agents were tested, divided into Comité de l'Antibiogramme de la Société Française de Microbiologie (CASFM)-recommended antibiotics (TABLE II) and additional antibiotics relevant for Human Medicine, Veterinary practice, or species differentiation (TABLE III).

Inhibition zones were measured, and isolates were classified as susceptible or resistant based on CASFM interpretive criteria. This allowed a comparative analysis of resistance patterns by species and animal origin.

Statistical analysis

Statistical analysis of the data was performed using SPSS Software (Statistical Package for Social Sciences), version 25.0 (IBM). Categorical variables (resistant vs. susceptible) were summarized as counts and percentages.

Comparisons between three groups of animal origin (broiler chicken, cattle, and sheep) were assessed to compare the antimicrobial resistance patterns according to *Campylobacter* species (*C. jejuni* vs. *C. coli*) using one-way analysis of variance (ANOVA). Post-hoc tests (unspecified, probably Tukey HSD) were used to identify differentiated pairs. A P-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Antimicrobial resistance rates of *Campylobacter jejuni* and *Campylobacter coli* isolates

The resistance rates obtained from *Campylobacter jejuni* and *Campylobacter coli* isolates from meat samples derived from broilers chickens, cattle and sheep, and tested against 14 antibiotics revealed a varied results depending on both *Campylobacter* species and animal origin (FIG. 1).

In broiler chickens isolates, *C. coli* marked the higher resistance levels than *C. jejuni* to the most of antibiotics, with 100 % of resistance rate to macrolides (Streptomycin, Erythromycin), Quinolones (Nalidixic acid), Fluoroquinolone (Ciprofloxacin) and Tetracycline. *C. coli* showed high resistance rates of 72 to 77 % to beta-lactams (Ampicillin, Amoxicillin-Clavulanic acid), and to cephalosporin of different generation (Cephalothin and Cefotaxime). Conversely, high susceptibility was notably observed for Kanamycin, Trimethoprim, and Chloramphenicol (100 %), with moderate rate to Gentamicin (50 %). *C. jejuni* displayed a high resistance rate representing 92 % to Quinolone (Nalidixic acid), Fluoroquinolone (Ciprofloxacin), and metronidazole antibiotic, 75 % to macrolide (Erythromycin) and around 69 % of Beta-lactams (Ampicillin, Amoxicillin-Clavulanic acid). Resistance rates to Cefotaxime, Streptomycin, Gentamicin and Tetracycline were 48, 46 and 43 % respectively. *C. jejuni* exhibited high susceptibility rate to Cefotaxime, Kanamycin, Trimethoprim, and Chloramphenicol (86 - 88 %), with lower rates to Cephalothin, Streptomycin, and Tetracycline (52 - 57 %).

In the other animal origin isolates, the total resistance (100 %) was just revealed to Quinolone (Nalidixic acid) in *C. coli* isolated from cattle, with high rates to Cephalosporin (Cefotaxime) 79

TABLE II

CASFM-recommended antimicrobial agents tested against thermotolerant *Campylobacter* isolates

Antibiotic	Disk content (µg)	Category / Use
AM (Ampicillin)	10	CASFM – human / veterinary β-lactam
AMC (Amoxicillin-Clavulanic acid)	30	CASFM – human β-lactam
CF (Cephalothin)	30	CASFM – human / species differentiation
CTX (Cefotaxime)	30	CASFM – human / β-lactam
GM (Gentamicin)	10	CASFM – human / veterinary aminoglycoside
K (Kanamycin)	30	CASFM – veterinary aminoglycoside

CASFM: Comité de l'Antibiogramme de la Société Française de Microbiologie.

TABLE III

Categorization of additional antibiotics tested by use

Antibiotic	Disk content (µg)	Category	Purpose / Notes
SP (Streptomycin)	10	Veterinary	Aminoglycoside for production animals
TM (Trimethoprim)	5	Veterinary / human	Blocks bacterial folate synthesis; inhibits bacterial growth
E (Erythromycin)	15	Human medicine	Human Macrolide therapy
CIP (Ciprofloxacin)	5	Human medicine	Fluoroquinolone for humans
TET (Tetracycline)	30	Human / veterinary	Widely used in humans and animals
C (Chloramphenicol)	30	Veterinary	Used in animals (where allowed)
NA (Nalidixic acid)	30	Species differentiation	Differentiation species (<i>C. jejuni</i> vs. <i>C. coli</i>)
MTR (Metronidazole)	5	Veterinary / experimental	Used for animal isolates or research

% and 57 % to Macrolide (Erythromycin). *C. coli* isolated from sheep species marked high rates of 70 % to both Cefotaxime and Metronidazole, with 50 % to Tetracycline. On the other hand, high susceptibility was marked to the most of antibiotics, especially Ampicillin, Amoxicillin-Clavulanic acid, Cefotaxime, Kanamycin, Trimethoprim, and Chloramphenicol (80 – 100 %) in a both of cattle and sheep origins.

For *C. jejuni* in cattle, resistance was noted to Metronidazole by 93 %, Ampicillin (67 %), and Streptomycin (60 %) with 47 % to both Erythromycin and Nalidixic acid. In contrast, full susceptibility was observed to Ciprofloxacin and Chloramphenicol. In sheep, *C. jejuni* isolates demonstrated high resistance rates of 63 % to Nalidixic Acid, 50 % to Chloramphenicol, and 44 % to Gentamicin. However, several antibiotics demonstrated significant efficacy, as reflected by their corresponding susceptibility rates such as Ciprofloxacin and Metronidazole (94 %), Tetracycline (81 %), and 75 % to most other tested antibiotics.

The antimicrobial resistance rates presented in FIG. 1 also highlight resistance across multiple antibiotic classes among several *Campylobacter* isolates, indicating the presence of multidrug resistant strains especially in broiler chickens' isolates.

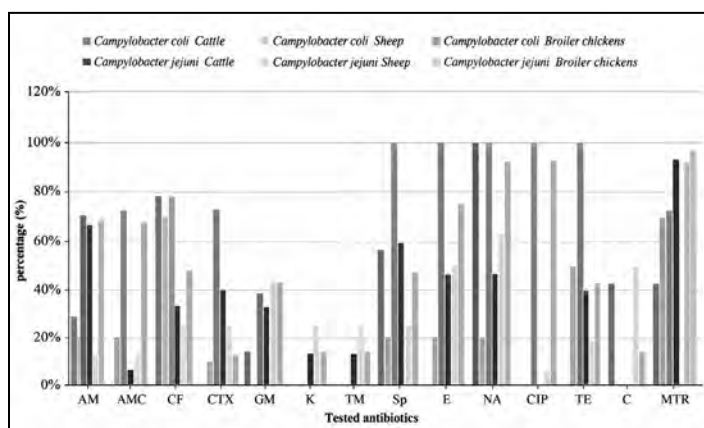


FIGURE 1. Resistance rates of *C. jejuni* and *C. coli* isolates to tested antibiotics in meat samples from different animal origins (broiler chickens, cattle and sheep).

Antimicrobial resistance is one of the most critical and urgent global health challenges, with significant implications for human healthcare systems as well as the veterinary and agriculture fields [24, 25]. Its rapid emergence and dissemination outpace development of newer antimicrobial agents [11, 26], largely attributable to their widespread use particularly in livestock production destined for human consumption [27, 28].

In Algeria, as in many other countries, antibiotics are routinely administered to food-producing animals (broiler chickens production as well as in cattle and sheep) mainly for prophylactic and therapeutic purposes, and in some farming system as growth promoters [29, 30].

National studies have reported many data on AMR profile variations and comparative analysis for *Salmonella* and *Escherichia coli* isolates from different animal origin. However, no studies have compared AMR profiles in *Campylobacter* isolates across animal sources, existing reports describe resistance patterns without animal interspecies comparisons.

In this context, the present study explored the antimicrobial resistant rate of *Campylobacter* isolates recovered from broiler chickens, cattle and sheep against 14 antibiotics. A comparative analysis was conducted to assess significant differences in resistance patterns according to the animal source of food products. Broiler chicken isolates exhibited the highest antimicrobial resistance rates among other animal sources, with a higher rate of *C. coli* than *C. jejuni*.

Broiler chickens Isolates generally have the highest resistance rates, highlighting the major role of intensive poultry farming in the selection and dissemination of resistant strains. Overall, *C. coli* has consistently demonstrated a higher resistance than *C. jejuni*, particularly in avian isolates. Alarming high resistance to quinolones, Fluoroquinolones, Macrolides, Tetracycline (100 %) and Beta-lactams (72 % -77 %) was observed in chickens *C. coli* isolates. Chicken *C. jejuni* showed 92 % resistance to Quinolones, Fluoroquinolones, and Metronidazole, 75 % to Macrolides, and 69 % to Beta-lactams.

These rates are of concern, given that these classes of antibiotics are widely used in veterinary and human medicine. Such resistances are generally associated with mutations targeting genes involved in the antibiotics mechanisms, as well as the presence of transmissible resistance genes, which are favoured by strong selection pressure [24].

High resistance to Fluoroquinolone, particularly to Nalidixic acid and Ciprofloxacin, suggests the intensive or inappropriate use of these antibiotics in poultry farming. Similarly, resistance to Erythromycin, the cornerstone antibiotic of human *Campylobacteriosis* treatment, poses a significant health risk. The finding agree with many study in Tunisia and also in Morocco which showed complete resistance of *C. coli* to Macrolide, Quinolone [25, 26], Kenya [11] and developing countries like China, united states [27, 28, 29]. However, some studies in Egypt, and Morocco showed that *C. jejuni* high resistant than *C. coli* in broiler isolates to the most antibiotic agents [30, 31].

Elevated resistance rates were also evident in isolates from cattle and sheep. Bovine *C. coli* isolates showed complete resistance to Quinolone, alongside high resistance to Cephalosporins (79 %) and Macrolides (59 %). Ovine *C. coli* also marked high rate to both Beta-lactams and Nitroimidazoles (70 %), with moderate resistance to Tetracycline. *C. jejuni* isolated from cattle demonstrated a substantial resistant to Nitroimidazoles (93 %), Beta-lactams (67 %) and Aminoglycosides (60 %), while notable resistance to Macrolides and Quinolones, consistently to results reported in recent study in Korea [32]. Ovine *C. jejuni*, displayed 63 % resistance to Quinolones, 50 % to Chloramphenicol, and moderate Aminoglycoside resistance.

These data provide substantial evidence that intensively farmed animals play a role in sustaining resistant *Campylobacter* isolates, aligning with Spanish and united states studies showing that ruminants (cattle and sheep) serve as key reservoir [33, 34]. This reflect irregular and inappropriate antibiotics use in the field, particularly prohibited Chloramphenicol administration [35, 36].

Regarding other animal origin isolates (cattle and sheep) high rates were also obtained, *C. coli* isolated from cattle showed a total resistance to Quinolone with increasing level

to Cephalosporin and Macrolide similarly (79 % and 59 % respectively), ovine *C. coli* also marked high rate to both Beta-lactams and Nitroimidazoles (70 %).

The statistical analysis of antimicrobial response profiles revealed significant association and differences among *Campylobacter* isolates according to animal origin for the majority of tested antibiotics (ANOVA, $P < 0.05$). This highlights considerable heterogeneity in resistance profiles depending on the origin of the isolates.

In the Beta-lactams and Cephalosporin antibiotic classes, significant differences were observed in isolates from broiler chickens compared to those from cattle and sheep. High resistance rates were observed for Ampicillin ($P = 0.023$), Amoxicillin-Clavulanic acid ($P = 0.004$), and Cefotaxime ($P < 0.001$).

Conversely, the bovine and ovine isolates exhibited significantly higher susceptibility rates (Ampicillin, $P = 0.002$; Amoxicillin-Clavulanic acid, $P < 0.001$; Cephalothin, $P = 0.045$), reaching up to 80 %. Notably, cefotaxime showed a marked variation ($P < 0.001$), with a high susceptibility of 87.69 % in broiler chicken isolates, predominantly *C. jejuni*. These resistances were primarily associated with *C. coli*, indicating a link between the dominant species in broiler chickens and elevated resistance levels.

Trimethoprim (antifolate class) and Kanamycin (aminoglycoside class) also exhibited significant differences ($P = 0.012$), with the highest resistance levels in broiler isolates. However, both antibiotics showed no significant variation ($P = 0.156$), displaying uniform susceptibility across all origins. Gentamicin displayed significant variation ($P = 0.023$), with full susceptibility in ovine *C. coli* and reduced rates in broiler chickens. These findings reinforce the association and concordance between poultry origin and the increased expression of resistance to these antibiotics (TABLE IV).

Furthermore, Macrolides (Streptomycin and Erythromycin), Fluoroquinolone (Ciprofloxacin) and Tetracycline showed significant differences characterized by extremely high resistance levels in broiler chickens' isolates, reaching 100 %, mainly linked with *C. coli*, reinforcing the association between avian origin, isolated species and the intensity of the observed resistance phenotype (TABLE V). For Quinolones, Nalidixic acid, although significant differences ($P = 0.002$) were observed between animal origins, with the highest resistance rates recorded in both bovine and broiler chickens' isolates. This indicates a shared high resistance across these origins, predominantly associated with *C. coli*.

TABLE IV
Analysis of Variance statistical results of antimicrobial resistance profiles of *Campylobacter* isolates in different animal origins

ATB	ANOVA P-value (between animal origins)	Significant differences ($P < 0.05$)	Resistance rate % (max by origin)
AM	0.023	Cattle vs. BC; Sheep vs. BC	BC (72.22 % <i>C. coli</i>)
AMC	0.004	Sheep vs. BC; Cattle vs. BC	BC (72.22 % <i>C. coli</i>)
CF	0.156	No significant	Cattle (78.57 % <i>C. coli</i>)
CTX	< 0.001	Cattle/Sheep vs. BC	BC (72.22 % <i>C. coli</i>)
GM	0.089	No significant	Sheep (43.80 % <i>C. jejuni</i>)
K	0.012	Cattle/Sheep vs. BC	BC (25 % <i>C. jejuni</i> Sheep)
TM	0.012	Cattle/Sheep vs. BC	BC (25 % <i>C. jejuni</i> Sheep)
SP	< 0.001	Cattle vs. BC; Sheep vs. BC	BC (100 % <i>C. coli</i>)
E	< 0.001	Cattle vs. BC/Sheep	BC (100 % <i>C. coli</i>)
NA	0.002	Cattle/Sheep vs. BC	Cattle (100 % <i>C. coli</i>)
CIP	< 0.001	Cattle/Sheep vs. BC	BC (100 % <i>C. coli</i>)
TE	0.001	Cattle vs. BC; Sheep vs. BC	BC (100 % <i>C. coli</i>)
C	< 0.001	Cattle vs. Sheep/BC	Sheep (50 % <i>C. jejuni</i>)
MTR	< 0.001	Cattle vs. BC; Sheep vs. BC	BC (93.30 % <i>C. jejuni</i>)

ANOVA: Analysis of variance, AMR: Antimicrobial resistance, ATB: Antibiotic, BC: broiler chickens, *C. jejuni*: *Campylobacter jejuni*, *C. coli*: *Campylobacter coli*, AM: Ampicillin, AMC: Amoxicillin-Clavulanic acid, CF: Cephalothin, CTX: Cefotaxime, GM: Gentamicin, K: Kanamycin, SP: Streptomycin, TM: Trimethoprim, E: Erythromycin, CIP: Ciprofloxacin, TET: Tetracycline, C: Chloramphenicol, NA: Nalidixic acid, MTR: Metronidazole.

Accordingly, susceptibility rates demonstrated significant variations ($P < 0.001$) to these antibiotic families, with bovine isolates showing complete susceptibility to Macrolides, Quinolones, and Fluoroquinolones (Nalidixic acid and Ciprofloxacin). *C. jejuni* isolates from ovine (80 %) and broiler chickens (93.75 %) demonstrated high susceptibility to these antibiotics. Tetracycline susceptibility differed significantly ($P = 0.001$), with complete sensitivity in bovine and ovine *C. coli* strains, while broiler chicken isolates showed reduced rates.

TABLE V
High resistant rates of antibiotics of *Campylobacter* isolates from different animal origin

Origin	Most resistant antibiotics (rate ≥ 90 %)	Isolates	Maximum rates
Cattle	NA (100 %)	<i>C. coli</i>	100 %
Sheep	NA (100 %), CIP (100 %), E (100 %), TE (100 %)	<i>C. coli</i> / <i>C. jejuni</i>	100 %
BC	Sp (100 %) E (100 %) CIP (100 %) TE (100 %) NA (92-100 %) MTR (93 %)	<i>C. coli</i> / <i>C. jejuni</i>	100 %

BC: broiler chickens, *C. jejuni*: *Campylobacter jejuni*, *C. coli*: *Campylobacter coli*, AM: Ampicillin, AMC: Amoxicillin-Clavulanic acid, CF: Cephalothin, CTX: Cefotaxime, GM: Gentamicin, K: Kanamycin, SP: Streptomycin, TM: Trimethoprim, E: Erythromycin, CIP: Ciprofloxacin, TET: Tetracycline, C: Chloramphenicol, NA: Nalidixic acid, MTR: Metronidazole.

Chloramphenicol ($P < 0.001$) displayed a distinct resistance profile, with the highest resistance observed in sheep isolates, predominantly associated with *C. jejuni*, and maximal susceptibility in *C. coli*. This pattern underscores a specific association between sheep origin, bacterial species and resistance phenotype. However, metronidazole ($P < 0.001$) showed significant differences between the three animal origins, with maximum resistance observed in broiler chickens' isolates, with peak resistance in broiler chicken isolates predominantly *C. jejuni* thereby underscoring a pronounced association between poultry origin and resistant profiles. Notably, susceptibility differed significantly ($P < 0.001$), particularly in ovine *C. jejuni* isolates, where it reached 93.75 % (TABLE VI).

TABLE VI

Analysis of variance statistical results of susceptibility levels in *Campylobacter* isolates from different animal origins

ATB	ANOVA P-value (between animal origins)	Significant differences (P < 0.05)	Susceptibility rate % (max by origin)
AM	0.002	Sheep vs. BC ; Cattle vs. BC	Sheep (80 % <i>C. coli</i>)
AMC	< 0.001	Cattle/Sheep vs. BC	Cattle (100 % <i>C. coli</i>)
CF	0.045	Cattle vs. Sheep/BC	Sheep (75 % <i>C. jejuni</i>)
CTX	< 0.001	Cattle/Sheep vs. BC	BC (87.69 % <i>C. jejuni</i>)
GM	0.023	Sheep vs. BC ; Cattle vs. BC	Sheep (100 % <i>C. coli</i>)
K	0.156	No significant (100%)	100 %
TM	0.156	No significant (100%)	100 %
SP	< 0.001	Cattle/Sheep vs. BC	Cattle (100 % <i>C. coli</i>)
E	< 0.001	Cattle/Sheep vs. BC	Cattle (100 % <i>C. coli</i>)
NA	< 0.001	Cattle vs. BC ; Sheep vs. BC	Sheep (80 % <i>C. coli</i>)
CIP	< 0.001	Cattle/Sheep vs. BC	BC (93.75 % <i>C. jejuni</i>)
TE	0.001	Cattle/Sheep vs. BC	Cattle (100 % <i>C. coli</i>)
C	0.034	Cattle vs. Sheep/BC	Sheep (100 % <i>C. coli</i>)
MTR	< 0.001	Sheep vs. BC; Cattle vs. BC	Sheep (93.75 % <i>C. jejuni</i>)

ANOVA: Analysis of variance, ATB: Antibiotic, BC: broiler chickens, *C. jejuni*: *Campylobacter jejuni*, *C. coli*: *Campylobacter coli*, AM: Ampicillin, AMC: Amoxicillin-Clavulanic acid, CF: Cephalothin, CTX: Cefotaxime, GM: Gentamicin, K: Kanamycin, SP: Streptomycin, TM: Trimethoprim, E: Erythromycin, CIP: Ciprofloxacin, TET: Tetracycline, C: Chloramphenicol, NA: Nalidixic acid, MTR: Metronidazole.

Conversely, no statistically significant variations were observed in resistance levels to Cephalothin (P = 0.156) and Gentamicin (P = 0.089) across host groups, indicating no clear association between animal origin and resistance to these specific antibiotics.

A detailed analysis of the resistance profiles according to isolated species reveals that broiler chickens' isolates demonstrated the highest resistance rates to most antibiotics, frequently associated with *C. coli* or *C. jejuni*. In contrast, bovine and ovine isolates displayed more heterogeneous resistance profiles, with resistance reaching 100 % for certain antibiotics contingent on species/origin combination (*C. coli* predominant in cattle; both *C. coli* and *C. jejuni* in sheep).

The analysis of variance was conducted to confirm that *Campylobacter* isolates broiler chickens represented higher resistance profiles to the most of antibiotic classes compared to bovine and ovine isolates with statistically significant differences (P < 0.05). These significant differences were particularly for Macrolides, Fluoroquinolone, Tetracycline and Nitroimidazoles in broiler chickens isolates showed the highest resistance.

However, bovine and ovine isolates exhibited elevated rate especially for Quinolones and their resistance were less extensive than observed in broiler chickens, these significant differences was also reported in other studies [37, 38] supporting the predominant role of broiler chickens as major reservoirs of antimicrobial resistant *Campylobacter* within the food production chain and underscore a discernible concordance between the poultry origin of the isolates and a stronger selection pressure driving the expression of resistance mechanisms specific to these

classes [39].

The present study also revealed high susceptibility rates marked in bovine and ovine isolates to the majority of antibiotics classes especially in *C. jejuni* following by *C. coli* (TABLE VII). The total rates were observed to Ciprofloxacin and Chloramphenicol in bovine *C. jejuni*, conversely reporting in other studies where *C. jejuni* in cattle represented a low susceptibility to Fluoroquinolone [40] and similar susceptibility reported in study of Tang, *et al.* [34].

TABLE VII
Highest rates of susceptibility to antibiotics of *Campylobacter* isolates from different animal origin

Origin	Antibiotics (susceptibility ≥ 80 %)	Isolates	Maximum rate
Cattle	AMC (100 %)	<i>C. coli</i>	100 %
	K (100 %)		
	TM (100 %)		
	SP (100 %)		
	E (100 %)		
	CIP (100 %)		
	TE (100 %)		
Sheep	K (100 %)	<i>C. coli</i> / <i>C. jejuni</i>	100 %
	TM (100 %)		
	GM (100 %)		
	CIP (100 %)		
	C (100 %)		
BC	K (100 %)	<i>C. jejuni</i>	100 %
	TM (100 %)		
	CTX (87- 88 %)		
	CIP (93 %)		

ANOVA: Analysis of variance, ATB: Antibiotic, BC: broiler chickens, *C. jejuni*: *Campylobacter jejuni*, BC: broiler chickens, *C. jejuni*: *Campylobacter jejuni*, *C. coli*: *Campylobacter coli*, AM: Ampicillin, AMC: Amoxicillin-Clavulanic acid, CF: Cephalothin, CTX: Cefotaxime, GM: Gentamicin, K: Kanamycin, SP: Streptomycin, TM: Trimethoprim, E: Erythromycin, CIP: Ciprofloxacin, TET: Tetracycline, C: Chloramphenicol, NA: Nalidixic acid, MTR: Metronidazole

Analysis of variance and interpretation of P-values revealed statistical consistency between resistance and sensitivity. The *C. coli* isolates from cattle and sheep were found to be highly and consistently susceptible to most of the antibiotics that were tested. *C. jejuni* dominated in broiler chicken isolates showed more heterogeneous and less favourable profiles. This variation based on animal provenance reinforces the robustness of the results and consolidates the conclusions drawn from the resistance analysis, which underscore the importance of considering the origin of isolates when evaluating resistance profiles.

CONCLUSION

This investigation demonstrates the highest resistance rates in *Campylobacter* isolates from broiler chickens especially in *Campylobacter coli* than *Campylobacter jejuni* including multidrug resistance observed in most of isolates. Comparative analysis of isolates from broiler chickens, cattle and sheep affirms that broiler chickens remain the primary reservoir of resistant *Campylobacter*, highlighting in the other side an emerging trend of antibiotic resistance trend among isolates from other livestock (cattle and sheep).

These findings underscore the critical need of continuous monitoring and implementation of prudent measures regarding antibiotic use and biosecurity in order to limit the spread of antibiotic resistance. Such actions are essential to protect animal health, the broader community, and the food supply under the one health approach, involving cooperation among various fields to optimize health outcomes for humans, animals, and the environment. It acknowledges the interconnectedness of human, animal, and environmental domains and recognizes their inherent interconnectedness.

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Conflicts of interest

The authors declare no conflicts of interest.

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