



## COMPARATIVE ANTIMICROBIAL RESISTANCE PROFILES OF ESCHERICHIA COLI FROM FATTENING PIGS AND CALVES IN SPAIN: AN ISOLATE-LEVEL MIC ANALYSIS

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### ABSTRACT

Antimicrobial resistance in food-producing animals is an important concern for animal and public health, as commensal bacteria may act as reservoirs in livestock production systems. In this study, antimicrobial resistance profiles of commensal *Escherichia coli* (*E. coli*) collected from fattening pigs and calves in Spain were compared using isolate-level minimum inhibitory concentration (MIC) data. All 340 isolates (170 pig derived and 170 calf derived) were tested and evaluated against 15 antimicrobial agents. Resistance was determined using antimicrobial-specific surveillance interpretive thresholds, while between-group differences were assessed using chi-square or Fisher's exact tests, odds ratios, multiple-testing correction, and multidrug-resistance analysis. The resistance levels to ampicillin, ciprofloxacin, trimethoprim, nalidixic acid, tetracycline, sulfamethoxazole and chloramphenicol were significantly higher for pig-derived isolates. The highest effect observed was for the drugs ciprofloxacin, ampicillin and trimethoprim. There were also significant variations in the source associated distributions for several agents in the MIC. Significantly more pig isolates had multidrug resistance (58.2%) than calf isolates (22.9%), and pigs had a higher burden of resistance. No resistance was observed against meropenem, colistin, amikacin, ceftazidime or tigecycline in either group. The results show a significantly higher antimicrobial-resistance burden in pig-derived *E. coli* and underscore the need to maintain isolate-level antimicrobial-resistance surveillance across livestock populations.

**Keywords:** antimicrobial resistance; *Escherichia coli*; fattening pigs; calves; minimum inhibitory concentration; multidrug resistance



## 1. Introduction

Antimicrobial resistance (AMR) is a significant issue at the animal health, food production and public health interface. Antimicrobial use in animals can lead to the selection for resistant bacteria. Food-producing animals can thus be a reservoir of antimicrobial-resistant microorganisms that can be transmitted directly to humans, via the food chain, and from animals to the environment. Monitoring of resistance in commensal and zoonotic bacteria is important as a source of data to help determine the patterns of resistance and detect changes that may need to be addressed. However, there has been significant variation in resistances within bacterial species, animal populations, countries, and classes of antimicrobials, emphasizing the importance of host- and species-specific monitoring (European Food Safety Authority & European Centre for Disease Prevention and Control, 2025).

*E. coli* is a commensal organism in the intestine of food-producing animals and is commonly used as an indicator organism to assess antimicrobial resistance in the microflora. Resistance in commensal *E. coli* may reflect antimicrobial exposure within livestock populations and may provide information on the wider antimicrobial-resistance reservoir in farms. Resistance has been reported for various antimicrobial classes in calves studies and highlight the importance of monitoring antimicrobial sensitivity in a phenotypical way in bovine production systems (Bortolami et al., 2024). Likewise, commensal *E. coli* from pigs is often resistant to tetracyclines, aminopenicillins, sulfonamides, trimethoprim and other antimicrobial agents (O'Neill et al., 2023).

Resistant *E. coli* can be affected by livestock production practices in terms of occurrence and distribution. The use of antimicrobials, production system and biosecurity measures are also linked to resistance in commensal bacterial populations from Spanish pig farms (Mencia-Ares et al., 2021). Resistance can also be a combination of resistances, rather than a resistance to a single drug. The multidrug resistance aspect of livestock-associated *E. coli* has been shown to be important, with characteristic patterns of resistance traits identified by cluster-based analyses (Hesp et al., 2021).

Phenotypic resistance surveillance commonly relies on minimum inhibitory concentration (MIC) measurements and epidemiological cut-off values. MIC-based approaches can give quantitative data on antimicrobial susceptibility and enable resistance distribution comparisons between populations of animals. In cattle and sheep, studies in Spain have also shown the value of MIC characterization in identifying resistant *E. coli* strains and description of resistance to epidemiologically significant antimicrobials (Tello et al., 2020). Similar long-term studies have also been performed in pigs and have identified resistance phenotypes and the presence of extended-spectrum beta-lactamase, AmpC and colistin-resistance determinants (Aguirre et al., 2020).

Comparisons are especially useful when made between different groups of livestock, as their exposure to antimicrobials, husbandry practices, age, production intensity and disease-management practices may be different. Existing surveillance has shown significant variations in the proportion of *E. coli* that are resistant within commensal *E. coli* populations from different livestock industries (Hesp et al., 2019). Routine monitoring of the population also indicates that healthy food producing animals may be shedding resistant *E. coli*, further highlighting the need for population level monitoring (Kidsley et al., 2018). However, European studies have also shown that farming practices can influence resistance profiles in porcine *E. coli* (Österberg et al., 2016). Accordingly, the present investigation examines isolate-level MIC data from commensal *E. coli* recovered from fattening pigs and calves in Spain. The objectives are:

To determine the antimicrobial resistance profiles of commensal *Escherichia coli* isolates from fattening pigs and calves using isolate-level minimum inhibitory concentration data.

To compare the prevalence of antimicrobial resistance between pig-derived and calf-derived *E. coli* isolates across the tested antimicrobial agents.

To evaluate multidrug resistance patterns and identify antimicrobials showing the strongest source-associated differences between pig and calf isolates.



## 2. Methodology

### 2.1 Study Design and Data Source

A secondary cross-sectional analysis was conducted using national antimicrobial resistance surveillance data reported for Spain for 2023. The source provided isolate-level antimicrobial susceptibility information for bacteria recovered from food-producing animals, including quantitative minimum inhibitory concentration (MIC) measurements expressed in mg/L and corresponding surveillance interpretive thresholds (Agencia Española de Consumo, Seguridad Alimentaria y Nutrición & Ministerio de Agricultura, Pesca y Alimentación, 2025). The individual bacterial isolate was considered the primary analytical unit, while antimicrobial-specific measurements represented susceptibility observations belonging to each isolate.

### 2.2 Study Population and Data Selection

The analysis was limited to only indicator commensal *Escherichia coli* samples that were routinely collected from caecal samples at slaughterhouses for antimicrobial resistance monitoring. Two groups of livestock were considered: fattening pigs and cattle less than one year old (calves). Records from targeted extended-spectrum beta-lactamase (ESBL) and carbapenemase (CPE) monitoring were not included, as these are specific parts of monitoring that may include isolates with specific resistance phenotypes, and may lead to biased estimates of the prevalence of routine resistance. The results of meat samples, other bacterial species, and confirmatory second-panel testing were also not included. A final analytical sample consisted of 340 *E. coli* isolates, comprising 170 pig-derived isolates and 170 calf-derived isolates. Susceptibility results were available for the same 15 antimicrobial agents for each isolate, resulting in 5,100 isolate–antimicrobial observations. To prevent multiply microbial measurements being considered as separate bacterial measurements, unique laboratory isolate identifiers were used.

### 2.3 MIC-Based Antimicrobial Resistance Assessment

The antimicrobial drugs included in the panel were amikacin, ampicillin, azithromycin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, colistin, gentamicin, meropenem, nalidixic acid, sulfamethoxazole, tetracycline, tigecycline, and trimethoprim. Quantitative MIC values and the corresponding surveillance interpretive thresholds were retained for each isolate–antimicrobial combination.

Each recorded MIC was compared to its microbiological threshold in order to determine the microbiological resistance. Isolates with MIC value > specified value were considered resistant or non-wild type isolates; those with MIC value at or below the specified value were considered at/below the surveillance threshold. These classifications are microbiological resistance and were not considered or interpreted as clinical treatment susceptibility.

Prevalence of resistance was determined for each antimicrobial separately and included the number and percentage of resistant isolates from pigs and calves. The MIC distributions were presented using the MIC<sub>50</sub> and MIC<sub>90</sub> values, which correspond to the concentration that inhibits 50% and 90% of the isolates, respectively. These measures enabled comparison of both categorical resistance prevalence and quantitative susceptibility distributions between the two livestock populations.

### 2.4 Multidrug Resistance and Resistance-Pattern Analysis

Multidrug resistance (MDR) was determined on the level of individual isolates and was defined as resistance to at least one antimicrobial agent in three or more antimicrobial classes. The antimicrobials tested were clustered into analytical antimicrobial categories for MDR assessment: aminoglycosides / amikacin, gentamicin; third generation cephalosporins / cefotaxime, ceftazidime; quinolones / ciprofloxacin, nalidixic acid; tetracycline-related / tetracycline, tigecycline. Ampicillin was grouped in the penicillin class, azithromycin belongs to the macrolide class, chloramphenicol to the phenicol class, colistin to the polymyxin class, meropenem to the carbapenem class, sulfamethoxazole to the sulfonamide class, and trimethoprim to the diaminopyrimidine class.

The count of resistant antimicrobial agents and resistant antimicrobial classes were determined for



each isolate. Isolates were classified as non-resistance, one class resistance, two classes resistance and three or more classes resistance. MDR prevalence was computed separately for pig-derived and calf-derived isolates. The most prevalent MDR patterns were also determined separately for the two livestock groups, and the MDR patterns were also built at the isolate level.

## 2.5 Statistical Analysis

Categorical variables were summarised in terms of frequencies and percentages. Pearson's chi-square was used to analyze the prevalence of resistance to each antimicrobial separately between isolates from pigs and isolates from calves. When expected cell frequencies were too small to use the chi-square approximation, Fisher's exact test was used.

For each difference identified as being associated with the source, the magnitude of the difference was expressed as odds ratios (ORs) with 95% confidence intervals and the calf-derived isolates were used as the reference group. An OR > 1 indicated higher odds of resistance among pig-derived isolates.

The false discovery rate was controlled using the Benjamini–Hochberg procedure, since 15 antimicrobial comparisons were done. Unadjusted p values and adjusted q values were derived and q values < 0.05 were deemed statistically significant. Because the distribution of resistance burdens was non-normally distributed and discrete, the number of antimicrobial classes with resistance per isolate was compared between the two groups of animals (pigs and calves) using the Mann–Whitney U test. Prevalence of MDR was compared between the two animal groups using chi square test or Fisher's exact test for statistical significance and the corresponding OR with 95% confidence interval was computed.

## 3. Results

### 3.1 Antimicrobial Resistance and MIC Profiles

The antimicrobial resistance of *Escherichia coli* was found to be markedly different between the isolates from fattening calves and pigs. Within pig-derived isolates, the highest prevalence of resistance was observed for ampicillin (67.6%), followed by tetracycline (59.4%), sulfamethoxazole (47.6%), trimethoprim (46.5%), ciprofloxacin (42.4%), and chloramphenicol (38.8%). Overall, 17.1% of pig isolates exhibited resistance to nalidixic acid, while resistance to azithromycin and gentamicin were both low at 2.4%. There was no resistance to amikacin, ceftazidime, colistin, meropenem or tigecycline in all of the pig isolates, while only one was resistant to cefotaxime.

The isolates from calves exhibited significantly lower resistance to all of the antimicrobial agents. The most common resistance phenotypes were tetracycline (31.8%), sulfamethoxazole (25.9%), chloramphenicol (20.0%), and ampicillin (14.7%). Trimethoprim, ciprofloxacin and nalidixic acid resistance was seen in 7.6%, 5.3% and 2.4% of the calf isolates respectively. Table 1 summarizes the antimicrobial-specific resistance frequencies together with MIC<sub>50</sub> and MIC<sub>90</sub> values for both livestock groups.

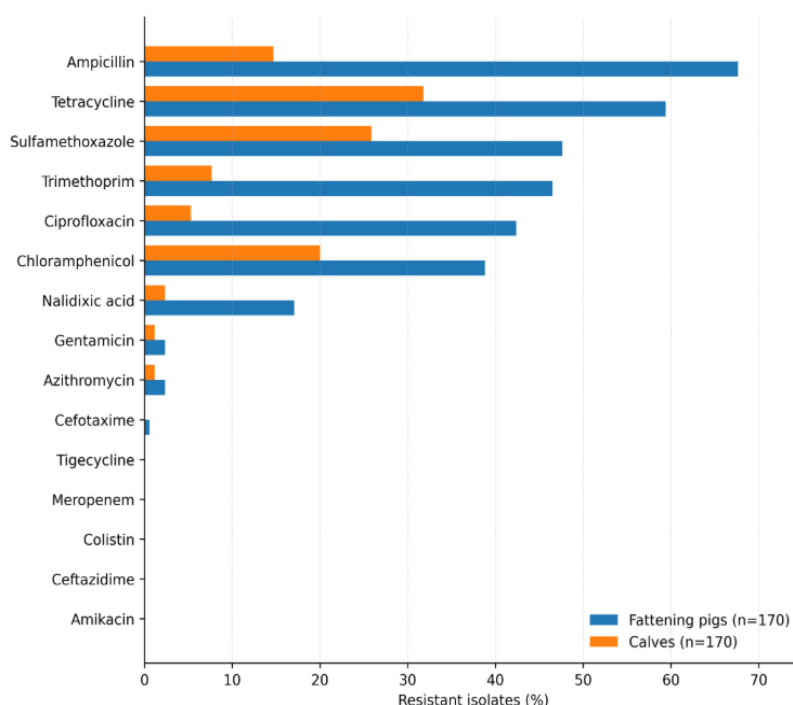
**Table 1. Antimicrobial resistance prevalence and MIC profiles of *Escherichia coli* isolates from fattening pigs and calves**

| Antimicrobial   | Pigs resistant, n/170 (%) | Pig MIC <sub>50</sub> (mg/L) | Pig MIC <sub>90</sub> (mg/L) | Calves resistant, n/170 (%) | Calf MIC <sub>50</sub> (mg/L) | Calf MIC <sub>90</sub> (mg/L) |
|-----------------|---------------------------|------------------------------|------------------------------|-----------------------------|-------------------------------|-------------------------------|
| Amikacin        | 0 (0.0)                   | 4                            | 4                            | 0 (0.0)                     | 4                             | 4                             |
| Ampicillin      | 115 (67.6)                | 32                           | 32                           | 25 (14.7)                   | 4                             | 32                            |
| Azithromycin    | 4 (2.4)                   | 4                            | 8                            | 2 (1.2)                     | 4                             | 8                             |
| Cefotaxime      | 1 (0.6)                   | 0.25                         | 0.25                         | 0 (0.0)                     | 0.25                          | 0.25                          |
| Ceftazidime     | 0 (0.0)                   | 0.25                         | 0.25                         | 0 (0.0)                     | 0.25                          | 0.25                          |
| Chloramphenicol | 66 (38.8)                 | 8                            | 64                           | 34 (20.0)                   | 8                             | 64                            |



|                  |            |       |      |           |       |      |
|------------------|------------|-------|------|-----------|-------|------|
| Ciprofloxacin    | 72 (42.4)  | 0.015 | 0.50 | 9 (5.3)   | 0.015 | 0.03 |
| Colistin         | 0 (0.0)    | 1     | 1    | 0 (0.0)   | 1     | 1    |
| Gentamicin       | 4 (2.4)    | 0.50  | 1    | 2 (1.2)   | 0.50  | 1    |
| Meropenem        | 0 (0.0)    | 0.03  | 0.03 | 0 (0.0)   | 0.03  | 0.03 |
| Nalidixic acid   | 29 (17.1)  | 4     | 64   | 4 (2.4)   | 4     | 4    |
| Sulfamethoxazole | 81 (47.6)  | 16    | 512  | 44 (25.9) | 16    | 512  |
| Tetracycline     | 101 (59.4) | 32    | 32   | 54 (31.8) | 2     | 32   |
| Tigecycline      | 0 (0.0)    | 0.25  | 0.25 | 0 (0.0)   | 0.25  | 0.25 |
| Trimethoprim     | 79 (46.5)  | 0.50  | 16   | 13 (7.6)  | 0.25  | 0.50 |

As shown in Figure 1, the greatest variations in resistance were seen between the animal groups for ampicillin, trimethoprim, ciprofloxacin and tetracycline. The percentage of resistant isolates was higher with pigs than with calves for all antimicrobial substances with moderate or high prevalence of resistance.



**Figure 1. Antimicrobial-specific resistance prevalence among *Escherichia coli* isolates from fattening pigs and calves**

### 3.2 Comparative Resistance Between Pig- and Calf-Derived Isolates

After multiple comparisons, seven antimicrobial agents were found to be significantly different between pig- and calf-derived isolates. Resistance was greatest for ampicillin with a difference of 52.9 percentage points in favour of the pig isolates. Trimethoprim showed a substantial difference of 38.8 percentage points, as well as significant differences in the other tested antimicrobial agents: ciprofloxacin (37.1 percentage points), tetracycline (27.6 percentage points), sulfamethoxazole (21.8 percentage points), chloramphenicol (18.8 percentage points), and nalidixic acid (14.7 percentage points).

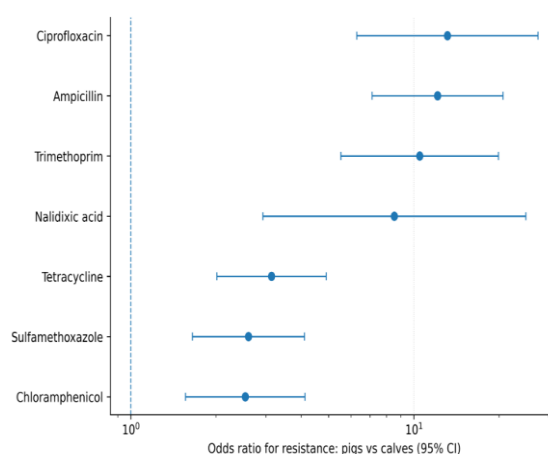
The largest estimated relative difference among the seven significant antimicrobials was observed for ciprofloxacin, which had 13.14 times greater odds of resistance in isolates from pigs than in isolates from calves, as reported in Table 2. High odds ratios were also noted for ampicillin (12.13), trimethoprim (10.48) and nalidixic acid (8.54). Other moderate but significant associations were seen for tetracycline, sulfamethoxazole, and chloramphenicol. After Benjamini–Hochberg correction all seven comparisons were statistically significant.

**Table 2. Antimicrobials showing significant differences in resistance between pig- and calf-derived isolates**

| Antimicrobial    | Difference in prevalence, percentage points | OR    | 95% CI     | p value                | Adjusted q value       |
|------------------|---------------------------------------------|-------|------------|------------------------|------------------------|
| Ampicillin       | 52.9                                        | 12.13 | 7.12–20.65 | $3.49 \times 10^{-23}$ | $5.24 \times 10^{-22}$ |
| Trimethoprim     | 38.8                                        | 10.48 | 5.52–19.90 | $7.83 \times 10^{-16}$ | $5.28 \times 10^{-15}$ |
| Ciprofloxacin    | 37.1                                        | 13.14 | 6.29–27.47 | $1.06 \times 10^{-15}$ | $5.28 \times 10^{-15}$ |
| Tetracycline     | 27.6                                        | 3.14  | 2.02–4.91  | $3.09 \times 10^{-7}$  | $1.16 \times 10^{-6}$  |
| Sulfamethoxazole | 21.8                                        | 2.61  | 1.65–4.11  | $3.16 \times 10^{-5}$  | $7.90 \times 10^{-5}$  |
| Chloramphenicol  | 18.8                                        | 2.54  | 1.56–4.13  | $1.40 \times 10^{-4}$  | $2.99 \times 10^{-4}$  |
| Nalidixic acid   | 14.7                                        | 8.54  | 2.93–24.86 | $4.65 \times 10^{-6}$  | $1.40 \times 10^{-5}$  |

OR > 1 indicates higher odds of resistance among pig-derived isolates. Adjusted q values were calculated using the Benjamini–Hochberg procedure.

Figure 2 presents the effect estimates and 95% confidence intervals for the seven significant antimicrobial comparisons. Although ciprofloxacin, ampicillin, trimethoprim, and nalidixic acid showed the largest odds ratios, the wider confidence interval for nalidixic acid reflected the smaller number of resistant isolates.

**Figure 2. Odds ratios and 95% confidence intervals for antimicrobial resistance in pig-derived relative to calf-derived Escherichia coli isolates**

### 3.3 Differences in MIC Distributions

Categorical resistance results were followed by changes in MIC distributions for some antimicrobials. Among pig isolates, the MIC<sub>50</sub> was 32 for ampicillin, but was 4 for calf isolates. There was an even greater difference in the central tendency with tetracycline, having an MIC<sub>50</sub> of 32 with pigs, and of 2 with calves. The difference in MIC<sub>50</sub> showed that higher MICs were seen in a much larger percentage of the pig isolates, but the MIC<sub>90</sub> remained the same for both groups (32).

The differences in upper MIC distributions were more noticeable for the quinolones. Ciprofloxacin had an MIC<sub>90</sub> of 0.50 among pig isolates compared with 0.03 among calf isolates, while nalidixic



acid had corresponding MIC<sub>90</sub> values of 64 and 4. The separation for trimethoprim was similar, an MIC<sub>90</sub> of 16 for pig-derived isolates and 0.50 for calf-derived isolates. However, chloramphenicol and sulfamethoxazole exhibited the same MIC<sub>50</sub> and MIC<sub>90</sub> values across groups, although there were marked differences in the percentage of strains with MICs above the surveillance interpretive thresholds. However, the MIC results showed that differences between groups were evident not only in resistance frequency but also in the susceptibility distributions for several antimicrobials.

### 3.4 Multidrug Resistance and Overall Resistance Burden

The overall antimicrobial resistance burden differed markedly between the two livestock groups. Resistance to at least one antimicrobial class was identified in 142 of 170 pig isolates, corresponding to 83.5%, compared with 61 of 170 calf isolates, corresponding to 35.9%. Consequently, no detected resistance was observed in only 16.5% of pig isolates but in 64.1% of calf isolates.

Multidrug resistance, defined as resistance to three or more antimicrobial classes, was detected in 99 pig-derived isolates and 39 calf-derived isolates. This corresponded to MDR prevalences of 58.2% and 22.9%, respectively. Pig-derived isolates had 4.68-fold higher odds of MDR than calf-derived isolates, with a 95% confidence interval of 2.93–7.49 and  $p < 0.001$ .

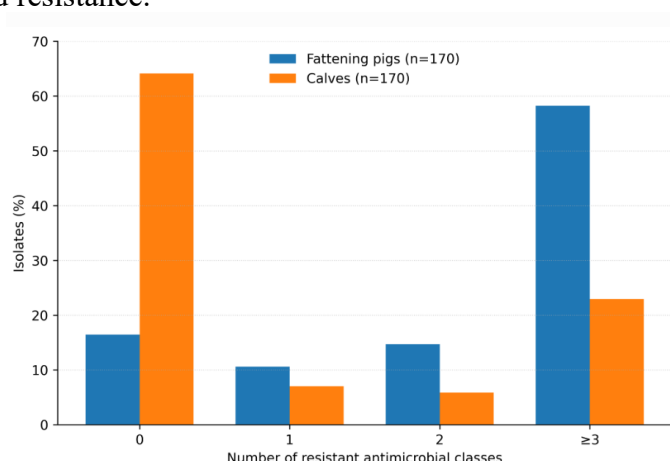
Table 3 further shows that 10.6% of pig isolates were resistant to one class and 14.7% to two classes, whereas the corresponding proportions among calves were 7.1% and 5.9%. The median resistance burden was three antimicrobial classes among pig isolates and zero among calf isolates. The distributions differed significantly according to the Mann–Whitney U test, with  $U = 22,400$  and  $p < 0.001$ .

**Table 3. Distribution of antimicrobial-class resistance and multidrug resistance**

| Resistance burden                   | Fattening pigs, n (%) | Calves, n (%) |
|-------------------------------------|-----------------------|---------------|
| No detected resistance              | 28 (16.5)             | 109 (64.1)    |
| Resistant to 1 class                | 18 (10.6)             | 12 (7.1)      |
| Resistant to 2 classes              | 25 (14.7)             | 10 (5.9)      |
| Resistant to $\geq 3$ classes (MDR) | 99 (58.2)             | 39 (22.9)     |
| Total                               | 170 (100.0)           | 170 (100.0)   |

MDR comparison: OR = 4.68, 95% CI = 2.93–7.49,  $p < 0.001$ . Median number of resistant classes: pigs = 3, calves = 0; Mann–Whitney  $U = 22,400$ ,  $p < 0.001$ .

The contrasting resistance burden is illustrated in Figure 3. Pig-derived isolates were concentrated in the multidrug-resistant category, whereas calf-derived isolates were predominantly represented by isolates without detected resistance.



**Figure 3. Distribution of pig- and calf-derived Escherichia coli isolates according to the number of resistant antimicrobial classes**



### 3.5 Predominant Multidrug Resistance Patterns

The composition of MDR profiles also differed between animal groups. Among the 99 MDR pig isolates, the most frequent exact resistance combination included ampicillin, chloramphenicol, ciprofloxacin, sulfamethoxazole, tetracycline, and trimethoprim and was identified in 15 isolates, corresponding to 15.2% of pig MDR isolates. The second most frequent pattern comprised ampicillin, sulfamethoxazole, tetracycline, and trimethoprim and occurred in 12 isolates.

Among the 39 MDR calf isolates, the predominant pattern consisted of simultaneous resistance to ampicillin, chloramphenicol, sulfamethoxazole, and tetracycline. This profile was detected in 15 isolates and accounted for 38.5% of calf MDR isolates. The combination of chloramphenicol, sulfamethoxazole, and tetracycline was the next most common profile and was observed in seven isolates. The most frequent MDR patterns are summarized in Table 4.

**Table 4. Predominant antimicrobial resistance combinations among multidrug-resistant isolates**

| Animal group   | Resistance combination                                                                        | n  | % of MDR isolates |
|----------------|-----------------------------------------------------------------------------------------------|----|-------------------|
| Fattening pigs | Ampicillin + chloramphenicol + ciprofloxacin + sulfamethoxazole + tetracycline + trimethoprim | 15 | 15.2              |
| Fattening pigs | Ampicillin + sulfamethoxazole + tetracycline + trimethoprim                                   | 12 | 12.1              |
| Fattening pigs | Ampicillin + chloramphenicol + sulfamethoxazole + tetracycline + trimethoprim                 | 7  | 7.1               |
| Calves         | Ampicillin + chloramphenicol + sulfamethoxazole + tetracycline                                | 15 | 38.5              |
| Calves         | Chloramphenicol + sulfamethoxazole + tetracycline                                             | 7  | 17.9              |
| Calves         | Ampicillin + sulfamethoxazole + tetracycline                                                  | 3  | 7.7               |

Taken together, the resistance profiles showed a consistently greater antimicrobial resistance burden among pig-derived *E. coli*. This difference was evident across individual antimicrobial agents, MIC distributions, overall resistance-class burden, and multidrug resistance patterns.

## 4. Discussion

The present analysis demonstrated clear differences in antimicrobial resistance between commensal *Escherichia coli* isolated from fattening pigs and calves. Across multiple antimicrobial classes, pig-derived isolates showed greater resistance than calf-derived isolates, with particularly high resistance to ampicillin, tetracycline, sulfamethoxazole, trimethoprim, ciprofloxacin, and chloramphenicol. Calf-derived isolates had lower resistance frequencies for most agents, although tetracycline, sulfamethoxazole, and chloramphenicol remained relatively prominent. The results suggest that, apart from the resistance to single antimicrobials, there are differences between the two livestock populations in the complexity of the resistance patterns.

The antimicrobial resistance seen in pig isolates is in line with the findings of high levels of antimicrobial resistance in pig *E. coli* previously reported. A study in Catalonia found high levels of resistance in both diarrheic and healthy weaned pigs, with multidrug-resistant (MDR) phenotypes often present for routine classes of antimicrobial agents (Garcias et al., 2024). In the present study, the same trend was observed with the slaughter-age fattening pigs, with more than two thirds of all isolates found to be ampicillin-resistant and resistance to tetracycline, sulfamethoxazole, trimethoprim, and ciprofloxacin being prevalent. The levels of these resistance frequencies indicate that resistant commensal *E. coli* are well entrenched in pig production systems and could be found even in clinically healthy pigs.



There were significant variations seen in the resistance to fluoroquinolones and quinolones. The odds of resistance to ciprofloxacin and nalidixic acid were significantly higher among pig-derived isolates than among calf-derived isolates, and the difference also was observed in the distributions of the MICs. This substantial difference in ciprofloxacin MIC<sub>90</sub> and nalidixic acid MIC<sub>90</sub> for pig isolates suggests that the difference was not confined to a minority of resistant isolates, but also included individuals in the upper part of the susceptibility profiles. Harmonized MIC-based methods have also been used in surveillance of porcine *E. coli* resistance in fattening pigs in Germany, showing variation in prevalence of resistance across subnational regions, in addition to geographic variation (Richter et al., 2025). This emphasizes the need for the consideration of livestock AMR in the context of certain production systems and regions, but not across all livestock populations.

The overall resistance of calf-derived isolates was lower, with about 1/3 resistant to tetracycline and about 1/4 resistant to sulfamethoxazole. Such findings remain epidemiologically significant because commensal *E. coli* may serve as reservoirs of resistance determinants even when resistance prevalence is lower than in other livestock groups. Longitudinal study of veal calves in France has shown that antimicrobial use during fattening can influence the prevalence and composition of antimicrobial-resistant *E. coli* over time (Gay et al., 2019). The cross-sectional study does not allow for exposure-response relationships or temporal changes to be quantified but the resistance found in calves warrants continued monitoring throughout the production cycle.

The MIC results were useful to give more information than just resistant or susceptible. There were significant differences between pigs and calves in MIC<sub>50</sub> values for ampicillin and tetracycline, and for ciprofloxacin, nalidixic acid and trimethoprim there were clear differences in the MIC<sub>90</sub> values. The changes suggest general variations in susceptibility distributions amongst animal sources. Similar findings have been reported in dairy calves, where antimicrobial exposure history has been associated with changes in phenotypic resistance and increased resistance in treated animals (Formenti et al., 2021). Thus, MIC distributions may offer a more precise picture of susceptibilities in the population than proportions of resistance.

Multidrug resistance was one of the greatest differences observed between the two livestock groups. The proportion of the pig-derived isolates that were resistant to three or more antimicrobial classes was greater than 50%, while the proportion of calf isolates resistant to 3 or more antimicrobial classes was less than one-quarter. The median number of classes of resistance in pig isolates was also greater and combinations of resistance to ampicillin, chloramphenicol, ciprofloxacin, sulfamethoxazole, tetracycline and trimethoprim were more complex. Previous studies have shown that variation in antimicrobial use and farm management is linked to different resistance patterns among the *E. coli* strains of swine, indicating that the burden of resistance may be a result of several interacting production level factors (Ketkhao et al., 2021). The present results thus confirm the advantages of the use of resistance combinations instead of a single antimicrobial agent.

One significant finding was that no resistance to meropenem, colistin, amikacin, ceftazidime, or tigecycline was found in either group of animals—with only a single pig isolate resistant to cefotaxime. These findings suggest that resistance was concentrated mainly among older and commonly used antimicrobial classes rather than being uniformly distributed across the full panel. This trend is similar to the one observed in Europe, where resistance in indicator *E. coli* from food-producing animals varies widely by antimicrobial class and resistance against those agents critical to public health is generally monitored separately due to its higher public-health relevance (European Food Safety Authority & European Centre for Disease Prevention and Control, 2023).

There are a number of caveats to take into account. This analysis is cross sectional and cannot address causal effects of antimicrobial use, management practices, and observed resistance. Farm-level exposure, treatment history, biosecurity and production system information was not available and could not be directly adjusted. Furthermore, the study was limited to routine commensal *E. coli* surveillance from two livestock populations and cannot be extended to other livestock species or pathogenic *E. coli*. However, the fact that the pig isolates and the calf isolates had an equal number of isolates, all isolates included MIC data, all isolates had the same MIC panel, and the isolates were



analyzed for resistance at the isolate level give a good foundation for comparative interpretation. Overall, the data indicated a significantly higher proportion of multidrug-resistant (MDR) and resistant *E. coli* associated with pigs, and there was an important proportion of resistant phenotypes associated with calf-derived *E. coli* that should be monitored.

## 5. Conclusion

Comparative study of commensal *Escherichia coli* from pigs and calves in fattening production showed that significant differences existed in the antimicrobial resistance of the strains from the two livestock groups. The antimicrobial resistance of pig-derived isolates was significantly higher against most of the antimicrobial agents tested, especially ampicillin, tetracycline, sulfamethoxazole, trimethoprim, nalidixic acid, chloramphenicol, and ciprofloxacin. These differences remained significant after correction for multiple comparisons and were supported by shifts in MIC distributions for several key antimicrobials. The significantly greater resistance prevalence to ciprofloxacin, ampicillin, trimethoprim and nalidixic acid in pig isolates suggests a high source-associated resistance burden.

Multidrug resistance further distinguished the two groups. Of the pig-derived isolates, over half were resistant to three or more antimicrobial classes, whereas less than one quarter of calf-derived isolates were resistant to three or more antimicrobial classes. The median number of resistant classes was also higher for pig isolates than for calf-derived isolates, as was the complexity of resistance combinations, reflecting broader co-occurrence of resistance traits. Calf derived isolates, in contrast, were more frequently found to have no detected resistance, though there were significant levels of resistance to tetracycline, sulfamethoxazole and chloramphenicol.

No resistance was detected to meropenem, colistin, amikacin, ceftazidime, or tigecycline in either group, suggesting that resistance was concentrated primarily among selected antimicrobial classes. Across the sample, the results confirm the importance of carry out isolate-level MIC surveillance to detect the resistance pattern and multi-drug resistance in livestock. Continued surveillance of commensal *E. coli* in food-producing animals, along with production system risk reduction measures and antimicrobial stewardship, is important for controlling the spread and potential carriage of antimicrobial resistance in and from animal production systems.

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