

## Supplemental Material

### Prevalence of cefotaxime-resistant *Escherichia coli* isolated from healthy cattle and sheep in Northern Spain: phenotypic and genome-based characterization of antimicrobial susceptibility

Maitane Tello<sup>1</sup>, Medelin Ocejó<sup>1</sup>, Beatriz Oporto<sup>1</sup>, Ana Hurtado<sup>1</sup>#

**Table S1.** Univariate and multivariate logistic regression analyses to assess association of different factors with prevalence of FOT-resistant *E. coli*.

| Variables                        | N   | Univariate        |                | Multivariate               |                |
|----------------------------------|-----|-------------------|----------------|----------------------------|----------------|
|                                  |     | OR (95% CI)       | <i>p</i> value | OR <sub>adj</sub> (95% CI) | <i>p</i> value |
| <b>Host Species</b>              |     |                   | 0.002          |                            | 0.002          |
| Ovine (Ref)                      | 114 |                   |                |                            |                |
| Bovine                           | 186 | 3.29 (1.47-7.35)  | 0.004          | 3.55 (1.57-8.04)           | 0.002          |
| <b>Farm management system</b>    |     |                   | < 0.001        |                            | < 0.001        |
| Sheep (Ref)                      | 114 |                   |                |                            |                |
| Beef Cattle                      | 104 | 1.41 (0.53-3.72)  | 0.488          | 1.65 (0.61-4.43)           | 0.324          |
| Dairy Cattle                     | 82  | 6.50 (2.77-15.27) | ≤ 0.001        | 6.11 (2.55-14.60)          | < 0.001        |
| <b>Sampling season</b>           |     |                   | 0.003          |                            | 0.022          |
| Winter (Ref)                     | 79  |                   |                |                            |                |
| Spring                           | 76  | 5.22 (1.43-19.16) | 0.003          | 5.19 (1.40-19.18)          | 0.014          |
| Summer                           | 45  | 5.48 (1.37-21.86) | 0.016          | 6.20 (1.53-25.14)          | 0.011          |
| Autumn                           | 100 | 6.73 (1.93-23.51) | 0.003          | 7.31 (2.07-25.78)          | 0.002          |
| <b>Geographical location</b>     |     |                   | 0.219          |                            |                |
| Oceanic (Ref)                    | 204 |                   |                | -                          | -              |
| Continental                      | 96  | 1.51 (0.79-2.91)  | 0.214          | -                          | -              |
| <b>Presence of other species</b> |     |                   | 0.026          |                            |                |
| Absence (Ref)                    | 156 |                   |                |                            |                |
| Presence                         | 139 | 0.47 (0.24-0.93)  | 0.030          | -                          | -              |
| <b>Herd size</b>                 |     |                   | 0.098          |                            |                |
| Small (Ref)                      | 84  |                   |                |                            |                |
| Medium                           | 109 | 0.67 (0.28-1.61)  | 0.375          | -                          | -              |
| Large                            | 96  | 1.58 (0.72-3.46)  | 0.254          | -                          | -              |
| <b>Year of sampling</b>          |     |                   | 0.709          |                            |                |
| 2014 (Ref)                       | 113 |                   |                |                            |                |
| 2015                             | 136 | 0.81 (0.40-1.63)  | 0.548          | -                          | -              |
| 2016                             | 51  | 1.13 (0.47-2.72)  | 0.784          | -                          | -              |

OR = Odds ratio, CI = confidence interval, Ref = reference category

**Table S2.** Overview of sequencing raw data and assembly for each sample.

| Sample ID | Raw Reads Stats |              | Assembly Stats    |               |                       |        |        |
|-----------|-----------------|--------------|-------------------|---------------|-----------------------|--------|--------|
|           | Total Reads     | Coverage (X) | Number of contigs | Genome Length | Average Contig Length | N50    | Mean Q |
| E_0670    | 10449667        | 316          | 233               | 5124332       | 377229                | 191127 | 35.9   |
| E_0684    | 11288346        | 341          | 426               | 5083178       | 304329                | 90141  | 36.0   |
| E_0685    | 11594106        | 350          | 426               | 5078030       | 204263                | 77542  | 36.1   |
| E_0688    | 10172991        | 307          | 323               | 5082618       | 693943                | 300597 | 36.1   |
| E_0696    | 9003830         | 272          | 310               | 5123383       | 313965                | 154413 | 36.0   |
| E_0699    | 9422908         | 285          | 2190              | 5730896       | 385298                | 154517 | 36.1   |
| E_0701    | 5512266         | 166          | 219               | 5002886       | 531328                | 184710 | 36.1   |
| E_0704    | 10114010        | 305          | 1238              | 5595899       | 538420                | 111013 | 36.1   |
| E_0706    | 9911660         | 299          | 344               | 5034410       | 375213                | 140612 | 36.1   |
| E_0708    | 8208039         | 248          | 165               | 5081399       | 303905                | 107823 | 36.1   |
| E_0713    | 8408546         | 254          | 70                | 4921505       | 617690                | 307619 | 36.1   |
| E_0715    | 9852072         | 298          | 3368              | 5956319       | 617690                | 197414 | 36.2   |
| E_0718    | 12075348        | 365          | 423               | 4830241       | 272238                | 67637  | 36.1   |
| E_0721    | 6704668         | 202          | 262               | 5013913       | 482556                | 180338 | 36.1   |
| E_0722    | 7537337         | 228          | 97                | 4970888       | 577591                | 229561 | 36.1   |
| E_0724    | 8511647         | 257          | 87                | 4760759       | 655571                | 235006 | 36.3   |
| E_0727    | 8576376         | 259          | 323               | 5131117       | 443643                | 160931 | 36.1   |
| E_0730    | 10255297        | 310          | 113               | 4710397       | 580758                | 141547 | 36.3   |
| E_0731    | 10392095        | 314          | 339               | 4949673       | 270088                | 127956 | 36.1   |
| E_0736    | 9425340         | 285          | 276               | 4982195       | 485749                | 153062 | 36.1   |
| E_0738    | 10739223        | 324          | 306               | 4820705       | 396548                | 148051 | 36.1   |
| E_0740    | 6058225         | 183          | 236               | 5096373       | 507731                | 192883 | 36.1   |
| E_0741    | 9450007         | 285          | 111               | 5062833       | 510463                | 192873 | 36.1   |
| E_0744    | 7767418         | 235          | 312               | 5133630       | 263722                | 112961 | 36.0   |
| E_0745    | 7547723         | 228          | 307               | 5130514       | 250954                | 101135 | 36.0   |
| E_0746    | 6795967         | 205          | 319               | 5172150       | 451627                | 140357 | 36.0   |
| E_0747    | 10466017        | 316          | 297               | 5128206       | 216206                | 101135 | 36.1   |
| E_0748    | 5771065         | 174          | 328               | 5443211       | 412575                | 192367 | 36.1   |
| E_0750    | 8771906         | 265          | 107               | 5128618       | 419721                | 186340 | 36.2   |
| E_0751    | 7626077         | 230          | 236               | 5163695       | 584633                | 184313 | 36.1   |
| E_0753    | 5694475         | 172          | 454               | 5273242       | 201498                | 83199  | 36.1   |
| E_0754    | 11306234        | 341          | 653               | 5324785       | 215631                | 80823  | 36.1   |
| E_0756    | 5210464         | 157          | 196               | 4932972       | 520968                | 156744 | 36.1   |
| E_0757    | 9543257         | 288          | 288               | 4958350       | 558770                | 175344 | 36.1   |
| E_0760    | 7766853         | 235          | 248               | 4971802       | 449956                | 143812 | 36.1   |
| E_0773    | 7039381         | 213          | 255               | 5021684       | 466502                | 174061 | 36.1   |
| E_0775    | 7622826         | 230          | 220               | 5010855       | 466502                | 184846 | 36.1   |
| E_0776    | 7099360         | 214          | 207               | 5428724       | 361337                | 153395 | 36.1   |
| E_0780    | 8518935         | 257          | 189               | 5239524       | 376463                | 170363 | 36.2   |
| E_0781    | 10143392        | 306          | 316               | 5019607       | 419338                | 159869 | 36.1   |
| E_0786    | 8427651         | 255          | 134               | 5194995       | 378190                | 154413 | 36.1   |

|        |          |     |     |         |        |        |      |
|--------|----------|-----|-----|---------|--------|--------|------|
| E_0787 | 7786516  | 235 | 127 | 5075828 | 353718 | 155777 | 36.1 |
| E_0789 | 11655146 | 352 | 418 | 5244828 | 657124 | 129801 | 36.1 |
| E_0790 | 9170976  | 277 | 337 | 5195112 | 657124 | 130992 | 36.1 |
| E_0792 | 7643794  | 231 | 179 | 5080889 | 481791 | 116603 | 36.1 |
| E_0795 | 7826957  | 236 | 115 | 5032537 | 581058 | 200385 | 36.1 |
| E_0796 | 7038812  | 213 | 319 | 5168309 | 410150 | 116911 | 36.1 |
| E_0798 | 8087201  | 244 | 189 | 5133473 | 371154 | 111618 | 36.1 |
| E_0802 | 10290772 | 311 | 403 | 5175436 | 430083 | 181418 | 36.1 |
| E_0803 | 6638566  | 200 | 222 | 4808631 | 389149 | 192918 | 36.1 |
| E_0806 | 5990319  | 181 | 216 | 5095474 | 522750 | 184343 | 36.1 |
| E_0811 | 8353535  | 252 | 115 | 4829516 | 507071 | 174720 | 36.2 |
| E_0812 | 9455310  | 286 | 179 | 5274771 | 452705 | 169624 | 36.1 |
| E_0817 | 7330179  | 221 | 153 | 4862709 | 333424 | 127725 | 36.3 |
| E_0818 | 8048331  | 243 | 237 | 4969464 | 617690 | 193574 | 36.1 |
| E_0820 | 7978446  | 241 | 308 | 4802893 | 272238 | 71218  | 36.1 |
| E_0821 | 9186047  | 277 | 319 | 5212464 | 410150 | 112991 | 36.1 |
| E_0822 | 8387153  | 253 | 279 | 5014700 | 574565 | 173293 | 36.1 |
| E_0825 | 7820435  | 236 | 251 | 5102287 | 313965 | 154413 | 36.1 |
| E_0826 | 7944475  | 240 | 343 | 5222936 | 384714 | 132476 | 36.1 |
| E_0828 | 8698058  | 263 | 361 | 5275849 | 415492 | 175277 | 36.1 |
| E_0832 | 7919339  | 239 | 178 | 5088265 | 273665 | 107823 | 35.9 |
| E_0833 | 10201182 | 308 | 382 | 5366702 | 538420 | 111889 | 36.1 |
| E_0834 | 8458453  | 255 | 348 | 5422047 | 735840 | 133443 | 36.1 |
| E_0836 | 7339805  | 222 | 243 | 5211472 | 522852 | 210039 | 36.1 |
| E_0837 | 8979130  | 271 | 312 | 5237591 | 522852 | 210039 | 36.1 |

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**Table S3.** Concordance tests between phenotypic antimicrobial susceptibility testing and WGS-based predicted antimicrobial resistance.

| Antimicrobial <sup>a</sup> | Sensitivity |            | Specificity |            | PPV <sup>b</sup> |            | NPV <sup>c</sup> |            | Cohen's kappa coefficient |            |                |                |
|----------------------------|-------------|------------|-------------|------------|------------------|------------|------------------|------------|---------------------------|------------|----------------|----------------|
|                            | %           | 95% CI     | %           | 95% CI     | %                | 95% CI     | %                | 95% CI     | $\kappa$                  | 95% CI     | <i>p</i> value | Interpretation |
| Tetracycline               | 100.0       | 92.6-100.0 | 100.0       | 82.4-100.0 | 100.0            | 92.6-100.0 | 100.0            | 82.4-100.0 | 1.00                      | 1.00-1.00  | <0.001         | Very Good      |
| Ciprofloxacin              | 100.0       | 87.9-100.0 | 100.0       | 90.8-100.0 | 100.0            | 87.9-100.0 | 100.0            | 90.8-100.0 | 1.00                      | 1.00-1.00  | <0.001         | Very Good      |
| Nalidixic acid             | 100.0       | 85.7-100.0 | 100.0       | 91.8-100.0 | 100.0            | 85.7-100.0 | 100.0            | 91.8-100.0 | 1.00                      | 1.00-1.00  | <0.001         | Very Good      |
| Chloramphenicol            | 100.0       | 83.9-100.0 | 84.8        | 71.8-92.4  | 74.1             | 55.3-86.8  | 100.0            | 91.0-100.0 | 0.77                      | 0.62-0.93  | <0.001         | Good           |
| Azithromycin <sup>d</sup>  | 100.0       | 61.0-100.0 | 100.0       | 94.0-100.0 | 100.0            | 61.0-100.0 | 100.0            | 94.0-100.0 | 100.0                     | 1.00-1.00  | <0.001         | Very Good      |
| Trimethoprim               | 100.0       | 90.1-100.0 | 100.0       | 89.0-100.0 | 100.0            | 90.1-100.0 | 100.0            | 89.0-100.0 | 1.00                      | 1.00-1.00  | <0.001         | Very Good      |
| Sulfamethoxazole           | 100.0       | 92.7-100.0 | 100.0       | 81.6-100.0 | 100.0            | 92.7-100.0 | 100.0            | 81.6-100.0 | 1.00                      | 1.00-1.00  | <0.001         | Very Good      |
| Gentamicin                 | 100.0       | 81.6-100.0 | 98.0        | 89.3-99.6  | 94.4             | 74.2-99.0  | 100.0            | 92.6-100.0 | 0.96                      | 0.89-1.00  | <0.001         | Very Good      |
| Cefoxitin                  | 85.7        | 60.1-96.0  | 100.0       | 93.1-100.0 | 100.0            | 75.8-100.0 | 96.3             | 87.5-99.0  | 0.90                      | 0.77-1.00  | <0.001         | Very Good      |
| Cefepime                   | 84.6        | 73.9-91.4  | 100.0       | 20.7-100.0 | 100.0            | 93.5-100.0 | 9.1              | 1.62-37.7  | 0.14                      | -0.11-0.39 | 0.024          | Poor           |

<sup>a</sup> No data are provided for cefotaxime and ampicillin since all isolates were phenotypically and genotypically resistant, or for ceftazidime, since all isolates carried at least one gene associated to ceftazidime resistance.

<sup>b</sup> PPV, Positive Predictive Value

<sup>c</sup> NPV, Negative Predictive Value

<sup>d</sup> No ECOFF value given by EUCAST; a MIC > 16 mg/L was used as resistance breakpoints reference as proposed (1, 2)

## REFERENCES

1. Clinical and Laboratory Standards Institute. 2015. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CSLI document M100-S25. Wayne.
2. Sjölund-Karlsson M, Joyce K, Blickenstaff K, Ball T, Haro J, Medalla FM, Fedorka-Cray P, Zhao S, Crump JA, Whichard JM. 2011. Antimicrobial susceptibility to azithromycin among *Salmonella enterica* isolates from the United States. *Antimicrob Agents Chemother* 55:3985–3989.

**Supplemental Data Set S1** (Excel file). List of genes detected by WGS (acquired resistance genes – ResFinder, and chromosomal point mutations – PointFinder) and chromosomal/plasmid location.