

**Table S2A. Assembly Quality and Core-Genome Locus Call Rates**

*Statistical comparisons between cohorts using two-tailed Mann-Whitney U test and Welch's unequal variances t-test.*

| Quality Metric                                   | Complete Cohort<br>(n=396) | Uncontaminated<br>(n=345) | Reference-Filtered<br>(n=51) | <i>p</i> -value (n=51 vs.<br>n=345) | <i>p</i> -value (n=396 vs.<br>n=345) |
|--------------------------------------------------|----------------------------|---------------------------|------------------------------|-------------------------------------|--------------------------------------|
| <b>Perc. Good Targets (%)</b><br>(Mean ± SD)     | 99.08 ± 0.66               | 99.13 ± 0.53              | 98.75 ± 1.19                 | <i>p</i> = 0.0140                   | <i>p</i> = 0.5190                    |
| <b>Median [IQR]</b>                              | 99.20 [98.70–99.43]        | 99.20 [98.70–99.50]       | 99.10 [98.60–99.40]          |                                     |                                      |
| <b>cgMLST Locus Call Rate</b><br>(%) (Mean ± SD) | 89.93 ± 3.89               | 89.97 ± 4.00              | 89.66 ± 3.11                 | <i>p</i> = 0.2824                   | <i>p</i> = 0.7778                    |
| <b>Median [IQR]</b>                              | 90.03 [87.84–90.91]        | 90.03 [87.90–90.97]       | 89.91 [87.68–90.60]          |                                     |                                      |
| <b>Called Core Loci Count</b><br>(Mean ± SD)     | 1434.45 ± 62.10            | 1435.09 ± 63.78           | 1430.14 ± 49.56              | <i>p</i> = 0.2824                   | <i>p</i> = 0.7778                    |
| <b>Median [IQR]</b>                              | 1436.00 [1401–1450]        | 1436.00 [1402–1451]       | 1434.00 [1398–1445]          |                                     |                                      |
| <b>Missing Distance Loci</b><br>(Mean ± SD)      | 160.56 ± 62.10             | 159.92 ± 63.79            | 164.86 ± 49.56               | <i>p</i> = 0.2824                   | <i>p</i> = 0.7778                    |
| <b>Genome Completeness</b><br>(%) (Mean ± SD)    | 99.99 ± 0.01               | 99.99 ± 0.01              | 100.00 ± 0.00                | <i>p</i> < 0.0001                   | <i>p</i> = 0.0220                    |

**Table S2B. Virulome Repertoire and Virulence Gene Frequencies**

*Comparison of key C. jejuni virulence factors across functional categories using two-tailed Fisher's exact test.*

| Functional Category         | Virulence Gene | Locus Tag | Ref-Filtered (n=51) | Uncontaminated (n=345) | Complete Cohort (n=396) | p-value (51 vs. 345) | p-value (396 vs. 345) |
|-----------------------------|----------------|-----------|---------------------|------------------------|-------------------------|----------------------|-----------------------|
| <b>Motility / Flagella</b>  | <i>flgB</i>    | Cj0527c   | 50 (98.0%)          | 345 (100.0%)           | 395 (99.7%)             | $p = 0.1288$         | $p = 1.0000$          |
|                             | <i>flgE</i>    | Cj0043    | 51 (100.0%)         | 345 (100.0%)           | 396 (100.0%)            | $p = 1.0000$         | $p = 1.0000$          |
|                             | <i>motA</i>    | Cj1023c   | 51 (100.0%)         | 344 (99.7%)            | 395 (99.7%)             | $p = 1.0000$         | $p = 1.0000$          |
| <b>Adherence / Invasion</b> | <i>cadF</i>    | Cj1478c   | 51 (100.0%)         | 343 (99.4%)            | 394 (99.5%)             | $p = 1.0000$         | $p = 1.0000$          |
|                             | <i>ciaB</i>    | Cj0914c   | 50 (98.0%)          | 342 (99.1%)            | 392 (99.0%)             | $p = 0.4252$         | $p = 1.0000$          |
|                             | <i>peb1A</i>   | Cj0921c   | 51 (100.0%)         | 345 (100.0%)           | 396 (100.0%)            | $p = 1.0000$         | $p = 1.0000$          |
|                             | <i>pldA</i>    | Cj0481    | 34 (66.7%)          | 198 (57.4%)            | 232 (58.6%)             | $p = 0.2264$         | $p = 0.7656$          |
| <b>Cytotoxic Toxin</b>      | <i>cdtA</i>    | Cj0498    | 51 (100.0%)         | 345 (100.0%)           | 396 (100.0%)            | $p = 1.0000$         | $p = 1.0000$          |
|                             | <i>cdtC</i>    | Cj0500    | 43 (84.3%)          | 289 (83.8%)            | 332 (83.8%)             | $p = 1.0000$         | $p = 1.0000$          |
| <b>Iron Acquisition</b>     | <i>cfrA</i>    | Cj0755    | 25 (49.0%)          | 196 (56.8%)            | 221 (55.8%)             | $p = 0.3649$         | $p = 0.8238$          |
|                             | <i>chuA</i>    | Cj1614    | 26 (51.0%)          | 166 (48.1%)            | 192 (48.5%)             | $p = 0.7648$         | $p = 0.9413$          |
| <b>Iron Acquisition</b>     | <i>feoB</i>    | Cj1398    | 46 (90.2%)          | 315 (91.3%)            | 361 (91.2%)             | $p = 0.7916$         | $p = 1.0000$          |
| <b>Stress Response</b>      | <i>sodB</i>    | Cj0169    | 50 (98.0%)          | 345 (100.0%)           | 395 (99.7%)             | $p = 0.1288$         | $p = 1.0000$          |
|                             | <i>katA</i>    | Cj1385    | 50 (98.0%)          | 344 (99.7%)            | 394 (99.5%)             | $p = 0.2413$         | $p = 1.0000$          |
|                             | <i>htrA</i>    | Cj1228c   | 51 (100.0%)         | 345 (100.0%)           | 396 (100.0%)            | $p = 1.0000$         | $p = 1.0000$          |
| <b>Chemotaxis</b>           | <i>cheA</i>    | Cj0284c   | 51 (100.0%)         | 341 (98.8%)            | 392 (99.0%)             | $p = 1.0000$         | $p = 1.0000$          |
|                             | <i>cheY</i>    | Cj1118c   | 51 (100.0%)         | 345 (100.0%)           | 396 (100.0%)            | $p = 1.0000$         | $p = 1.0000$          |

*Global locus-by-locus call rate correlation across all 1,595 loci: Pearson  $r = 0.9918$  ( $p < 10^{-15}$ ).*

**Table S2C. Host and Lineage Distribution Across Cohorts**

*$\chi^2$ -square test of independence for epidemiological and clonal structure.*

| Category                      | Stratification     | Ref-Filtered (n=51) | Uncontaminated (n=345) | Total (n=396) | $\chi^2$ Test Statistic               | p-value      |
|-------------------------------|--------------------|---------------------|------------------------|---------------|---------------------------------------|--------------|
| <b>Host Species</b>           | Bovine             | 5 (9.8%)            | 42 (12.2%)             | 47 (11.9%)    | $\chi^2 = 0.8741$                     | $p = 0.9282$ |
|                               | Broiler            | 8 (15.7%)           | 64 (18.6%)             | 72 (18.2%)    |                                       |              |
|                               | Human              | 15 (29.4%)          | 95 (27.5%)             | 110 (27.8%)   |                                       |              |
|                               | Turkey             | 2 (3.9%)            | 18 (5.2%)              | 20 (5.1%)     |                                       |              |
|                               | Yellow-legged gull | 21 (41.2%)          | 126 (36.5%)            | 147 (37.1%)   |                                       |              |
| <b>Major Clonal Complexes</b> | ST-1275 complex    | 12 (23.5%)          | 63 (18.3%)             | 75 (18.9%)    | $\chi^2 = 15.3196$<br>(for major CCs) | $p = 0.4287$ |
|                               | ST-21 complex      | 7 (13.7%)           | 51 (14.8%)             | 58 (14.6%)    |                                       |              |
|                               | ST-353 complex     | 6 (11.8%)           | 46 (13.3%)             | 52 (13.1%)    |                                       |              |
|                               | ST-443 complex     | 4 (7.8%)            | 17 (4.9%)              | 21 (5.3%)     |                                       |              |
|                               | ST-45 complex      | 3 (5.9%)            | 9 (2.6%)               | 12 (3.0%)     |                                       |              |
| <b>Major Clonal Complexes</b> | ST-42 complex      | 2 (3.9%)            | 10 (2.9%)              | 12 (3.0%)     |                                       |              |
|                               | ST-206 complex     | 1 (2.0%)            | 11 (3.2%)              | 12 (3.0%)     |                                       |              |
|                               | ST-52 complex      | 2 (3.9%)            | 9 (2.6%)               | 11 (2.8%)     |                                       |              |
|                               | ST-574 complex     | 3 (5.9%)            | 3 (0.9%)               | 6 (1.5%)      |                                       |              |
|                               | UNDEF              | 9 (17.6%)           | 52 (15.1%)             | 61 (15.4%)    |                                       |              |

**Table S3. Comprehensive statistical profiling and prevalence of 84 virulence-associated determinants across human, livestock, and yellow-legged gull reservoirs in Croatia (N = 396).** Categorical comparisons across the five host reservoirs were evaluated using two-tailed Pearson's Chi-square ( $\chi^2$ ) tests or Fisher's exact tests. *p*-values were adjusted for multiple testing across all 84 screened loci using the Benjamini–Hochberg False Discovery Rate (FDR) procedure ( $\alpha = 0.05$ , *q*-value).

| Function                             | Gene          | Total (396) % (n)<br>[95% CI]        | Bovine<br>(47) % (n) | Broiler<br>(72) % (n) | Human<br>(110) % (n) | Turkey<br>(20) % (n) | Gull<br>(147) % (n) | $\chi^2$<br>(df=4) | Nom. <i>p</i> -<br>value   | FDR <i>q</i> -<br>value | Reservoir<br>Association / Host<br>Distribution                                      |
|--------------------------------------|---------------|--------------------------------------|----------------------|-----------------------|----------------------|----------------------|---------------------|--------------------|----------------------------|-------------------------|--------------------------------------------------------------------------------------|
| Immune<br>Evasion (EVA)              | <i>wlaN</i>   | <b>18.43%</b> (73)<br>[14.87–22.59]  | 29.79%<br>(14)       | 22.22%<br>(16)        | 24.55%<br>(27)       | 35.00%<br>(7)        | 6.12%<br>(9)        | <b>25.92</b>       | <b>3.3 10<sup>-5</sup></b> | <b>0.0013</b>           | Significant<br>restriction; depleted<br>in wild gulls,<br>enriched in ST-21<br>hosts |
| LOS<br>Biosynthesis<br>& Sialylation | <i>cstIII</i> | <b>18.69%</b> (74)<br>[15.11–22.87]  | 12.77%<br>(6)        | 33.33%<br>(24)        | 25.45%<br>(28)       | 20.00%<br>(4)        | 8.16%<br>(12)       | <b>25.30</b>       | <b>4.4 10<sup>-5</sup></b> | <b>0.0013</b>           | Significant<br>enrichment in broiler<br>and human clinical<br>strains                |
| LOS<br>Sialylation<br>Cluster        | <i>neuA1</i>  | <b>25.51%</b> (101)<br>[21.43–30.07] | 21.28%<br>(10)       | 38.89%<br>(28)        | 33.64%<br>(37)       | 25.00%<br>(5)        | 14.29%<br>(21)      | <b>20.80</b>       | <b>0.00035</b>             | <b>0.0039</b>           | Enriched in poultry<br>and human clinical<br>isolates                                |
|                                      | <i>neuB1</i>  | <b>26.01%</b> (103)<br>[21.90–30.60] | 23.40%<br>(11)       | 40.28%<br>(29)        | 33.64%<br>(37)       | 25.00%<br>(5)        | 14.29%<br>(21)      | <b>21.62</b>       | <b>0.00024</b>             | <b>0.0031</b>           | Enriched in poultry<br>and human clinical<br>isolates                                |
|                                      | <i>neuC1</i>  | <b>26.01%</b> (103)<br>[21.90–30.60] | 23.40%<br>(11)       | 40.28%<br>(29)        | 33.64%<br>(37)       | 25.00%<br>(5)        | 14.29%<br>(21)      | <b>21.62</b>       | <b>0.00024</b>             | <b>0.0031</b>           | Enriched in poultry<br>and human clinical<br>isolates                                |
| Capsular<br>Polysaccharid<br>e (CPS) | <i>fcl</i>    | <b>11.36%</b> (45)<br>[8.55–14.93]   | 6.38% (3)            | 26.39%<br>(19)        | 13.64%<br>(15)       | 5.00%<br>(1)         | 4.76%<br>(7)        | <b>25.02</b>       | <b>5.0 10<sup>-5</sup></b> | <b>0.0013</b>           | Significant poultry-<br>associated lineage<br>enrichment                             |

| Function                      | Gene                                                                                                                                                                                                                                                                                                                          | Total (396) % (n)<br>[95% CI] | Bovine (47) % (n) | Broiler (72) % (n) | Human (110) % (n) | Turkey (20) % (n) | Gull (147) % (n) | $\chi^2$ (df=4) | Nom. <i>p</i> -value | FDR <i>q</i> -value | Reservoir Association / Host Distribution         |
|-------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|-------------------|--------------------|-------------------|-------------------|------------------|-----------------|----------------------|---------------------|---------------------------------------------------|
| Capsular Polysaccharide (CPS) | <i>glf</i>                                                                                                                                                                                                                                                                                                                    | 10.86% (43)<br>[8.11–14.39]   | 4.26% (2)         | 25.00% (18)        | 13.64% (15)       | 5.00% (1)         | 4.76% (7)        | 24.22           | 7.2 10 <sup>-5</sup> | 0.0014              | Significant poultry-associated lineage enrichment |
|                               | <i>kpsC</i> ,<br><i>kpsD</i> ,<br><i>kpsF</i> ,<br><i>kpsS</i> ,<br><i>kpsT</i>                                                                                                                                                                                                                                               | 100.00% (396)<br>[99.04–100]  | 100% (47)         | 100% (72)          | 100% (110)        | 100% (20)         | 100% (147)       | 0.00            | 1.000                | 1.000               | Conserved capsule backbone core                   |
| Motility Loci (MOT)           | <i>flgE2</i>                                                                                                                                                                                                                                                                                                                  | 6.06% (24)<br>[4.03–8.99]     | 0.00% (0)         | 8.33% (6)          | 10.00% (11)       | 10.00% (2)        | 3.40% (5)        | 8.16            | 0.086                | 0.448               | Lineage-associated structural variant             |
|                               | <i>flaA</i> ,<br><i>flaB</i> ,<br><i>flaC</i> ,<br><i>flgA</i> -D,<br><i>flgE</i> ,<br><i>flgG</i> -K,<br><i>flgP</i> -S,<br><i>flhA</i> -B,<br><i>flhF</i> ,<br><i>fliA</i> ,<br><i>fliF</i> ,<br><i>fliH</i> -N,<br><i>fliP</i> -R,<br><i>fliS</i> ,<br><i>fliW</i> ,<br><i>fliY</i> ,<br><i>motA</i> ,<br><i>motB</i> (39) | 100.00% (396)<br>[99.04–100]  | 100% (47)         | 100% (72)          | 100% (110)        | 100% (20)         | 100% (147)       | 0.00            | 1.000                | 1.000               | Ubiquitous flagellar basal body & motor core      |

| Function                             | Gene                                                                  | Total (396) % (n)<br>[95% CI]        | Bovine<br>(47) % (n) | Broiler<br>(72) % (n) | Human<br>(110) % (n) | Turkey<br>(20) % (n) | Gull<br>(147) % (n) | $\chi^2$<br>(df=4) | Nom. <i>p</i> -value | FDR q-value | Reservoir<br>Association / Host<br>Distribution |
|--------------------------------------|-----------------------------------------------------------------------|--------------------------------------|----------------------|-----------------------|----------------------|----------------------|---------------------|--------------------|----------------------|-------------|-------------------------------------------------|
| <b>Capsular Polysaccharide (CPS)</b> | <i>flgG2</i> ,<br><i>flhG</i> ,<br><i>fliI</i>                        | <b>98.74–99.75%</b><br>(391–395)     | 100% (47)            | 100% (72)             | 99.09% (109)         | 100% (20)            | 98.64% (145)        | 1.84               | 0.765                | 0.950       | Highly conserved motility accessory loci        |
| <b>Chemotaxis (CHEM)</b>             | <i>cheA</i> ,<br><i>cheV</i> ,<br><i>cheW</i> ,<br><i>cheY</i><br>(4) | <b>100.00%</b> (396)<br>[99.04–100]  | 100% (47)            | 100% (72)             | 100% (110)           | 100% (20)            | 100% (147)          | 0.00               | 1.000                | 1.000       | Universal enteric navigation core               |
| <b>Adhesion (ADH)</b>                | <i>cadF</i> ,<br><i>jlpA</i> ,<br><i>pebA</i><br>(3)                  | <b>100.00%</b> (396)<br>[99.04–100]  | 100% (47)            | 100% (72)             | 100% (110)           | 100% (20)            | 100% (147)          | 0.00               | 1.000                | 1.000       | Essential mucosal colonization core             |
| <b>Invasion (INV)</b>                | <i>ciaB</i> ,<br><i>eptC</i>                                          | <b>100.00%</b> (396)<br>[99.04–100]  | 100% (47)            | 100% (72)             | 100% (110)           | 100% (20)            | 100% (147)          | 0.00               | 1.000                | 1.000       | Universally conserved invasion apparatus        |
|                                      | <i>ciaC</i>                                                           | <b>98.23%</b> (389)<br>[96.38–99.16] | 97.87% (46)          | 98.61% (71)           | 99.09% (109)         | 100% (20)            | 97.96% (144)        | 0.74               | 0.946                | 0.985       | High conservation across all reservoirs         |
| <b>Cytotoxic (TOX)</b>               | <i>cdtC</i>                                                           | <b>100.00%</b> (396)<br>[99.04–100]  | 100% (47)            | 100% (72)             | 100% (110)           | 100% (20)            | 100% (147)          | 0.00               | 1.000                | 1.000       | Universal holotoxin subunit                     |
|                                      | <i>cdtA</i> ,<br><i>cdtB</i>                                          | <b>97.73%</b> (387)<br>[95.73–98.81] | 97.87% (46)          | 98.61% (71)           | 99.09% (109)         | 100% (20)            | 95.92% (141)        | 2.52               | 0.641                | 0.950       | Highly conserved holotoxin complex              |
| <b>Core LPS/LOS Backbone</b>         | <i>gmhA</i> ,<br><i>gmhA2</i> ,<br><i>gmB</i> ,<br><i>hldD</i> ,      | <b>99.75–100.00%</b><br>(395–396)    | 100% (47)            | 100% (72)             | 100% (110)           | 100% (20)            | 99.32% (146)        | 1.70               | 0.791                | 0.950       | Structural cell-wall integrity core             |

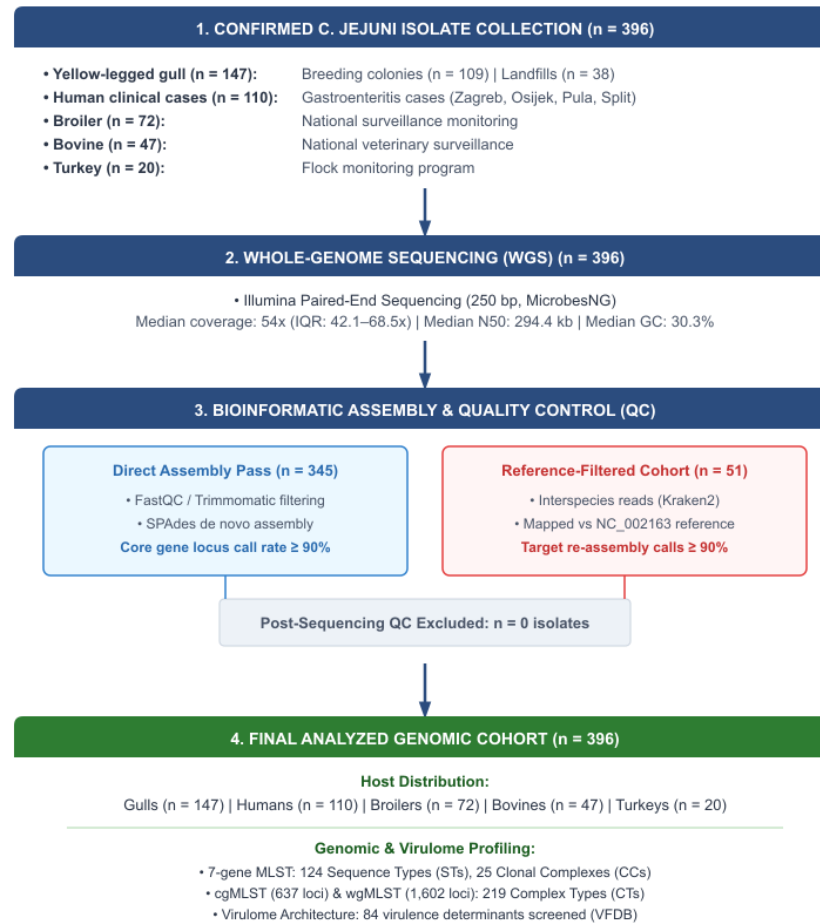
| Function                       | Gene                                                                  | Total (396) % (n)<br>[95% CI] | Bovine<br>(47) % (n) | Broiler<br>(72) %<br>(n) | Human<br>(110) %<br>(n) | Turkey<br>(20) %<br>(n) | Gull<br>(147) %<br>(n) | $\chi^2$<br>(df=4) | Nom. <i>p</i> -<br>value | FDR q-<br>value | Reservoir<br>Association / Host<br>Distribution |
|--------------------------------|-----------------------------------------------------------------------|-------------------------------|----------------------|--------------------------|-------------------------|-------------------------|------------------------|--------------------|--------------------------|-----------------|-------------------------------------------------|
|                                | <i>hldE</i> ,<br><i>cysC</i> ,<br><i>waaC</i> ,<br><i>waaF</i><br>(8) |                               |                      |                          |                         |                         |                        |                    |                          |                 |                                                 |
| Core LPS/LOS<br>Backbone       | <i>waaV</i>                                                           | 95.45% (378)<br>[92.90–97.13] | 95.74%<br>(45)       | 97.22%<br>(70)           | 95.45%<br>(105)         | 95.00%<br>(19)          | 94.56%<br>(139)        | 0.86               | 0.930                    | 0.985           | Conserved LOS<br>transferase                    |
| Type IV<br>Secretion<br>(T4SS) | <i>virB4</i> ,<br><i>virB8</i> ,<br><i>virB9</i> ,<br><i>virB10</i>   | 1.26% (5) [0.54–<br>2.92]     | 0.00% (0)            | 2.78%<br>(2)             | 0.00%<br>(0)            | 0.00%<br>(0)            | 2.04%<br>(3)           | 3.86               | 0.425                    | 0.900           | Plasmid pVir locus;<br>rare across all hosts    |
|                                | <i>virD4</i>                                                          | 0.51% (2) [0.14–<br>1.82]     | 0.00% (0)            |                          |                         |                         |                        |                    |                          |                 |                                                 |

### Overall Virulome Functional Burden Across Reservoirs

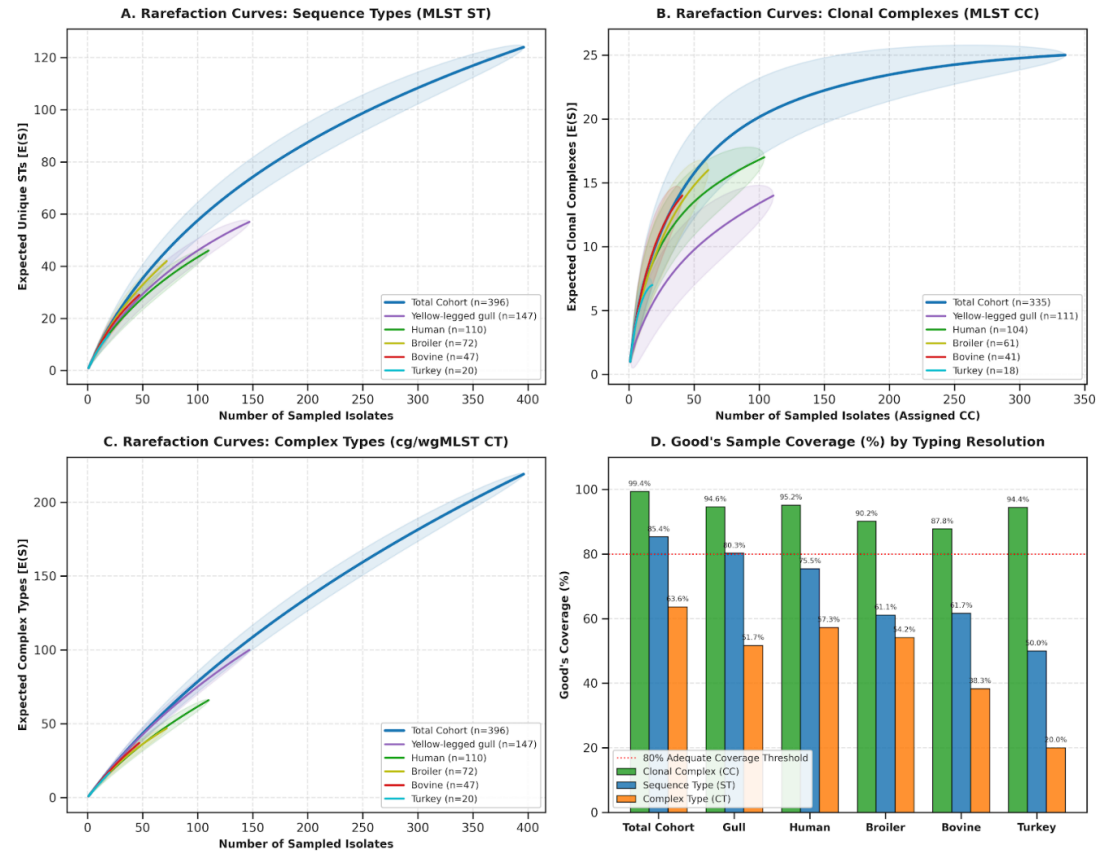
- **Broiler Cohort (n = 72):** 83.55% overall locus test positivity (n = 5 053 / 6,048 locus tests).
- **Human Cohort (n = 110):** 81.71% overall locus test positivity (n = 7 550 / 9 240 locus tests).
- **Turkey Cohort (n = 20):** 81.43% overall locus test positivity (n = 1 368 / 1 680 locus tests).
- **Bovine Cohort (n = 47):** 80.22% overall locus test positivity (n = 3 167 / 3 948 locus tests).
- **Yellow-Legged Gull Cohort (n = 147):** 79.02% overall locus test positivity (n = 9 757 / 12 348 locus tests).

Statistical Comparison (Broiler vs. Gull):  $\chi^2 = 54.21$ , df = 1, Benjamini–Hochberg FDR-adjusted  $p < 0.0001$ .

### Study Sample Formation and Bioinformatic Quality Control Flowchart



**Supplementary Figure S1.** Flowchart of *Campylobacter jejuni* isolate cohort formation and downstream whole-genome sequencing (WGS) genomic profiling



**Supplementary Figure S2.** Analytical sample-based rarefaction curves and sampling adequacy assessments for *Campylobacter jejuni* across five host reservoirs in Croatia (2021–2025). (A) Expected Sequence Type (ST) richness [E(S)] plotted against the number of sampled isolates across hosts. Shaded areas represent 95% confidence intervals calculated using analytical variance formulations (Heck et al., 1975). (B) Rarefaction curves for MLST Clonal Complexes (CC), showing complete plateauing and asymptotic saturation of circulating clonal lineages (Good's coverage > 90% in all major hosts). (C) Rarefaction curves for 1,602-locus cg/wgMLST Complex Types (CT), illustrating ongoing fine-scale genomic diversification. (D) Good's sample coverage (%) across host reservoirs for CC, ST, and CT typing resolutions. The red dotted line indicates the 80% coverage threshold for representative epidemiological sampling.