

Supplementary files

Supplementary Table S1: β -lactamase genes belonging to *bla*_{CTX-M} and *bla*_{TEM} type detected by PCR, Sanger sequencing and WGS from ESBL *E. coli* (n=31) sampled from healthy pigs and farm environment, northern Italy. The sample G1PAE6 was only tested through PCR and Sanger sequencing. *S1FHE2 resulted positive for *bla*_{CTX-M-14} by WGS and not by PCR.

| Sample | Source | <i>bla</i> _{CTX-M} | <i>bla</i> _{TEM} |
|---------|------------------|--------------------------------|-----------------------------|
| G1PAE2 | weaned pig | <i>bla</i> _{CTX-M-1} | <i>bla</i> _{TEM-1} |
| G1PAE3 | weaned pig | <i>bla</i> _{CTX-M-1} | <i>bla</i> _{TEM-1} |
| G1PAE4 | weaned pig | <i>bla</i> _{CTX-M-1} | <i>bla</i> _{TEM-1} |
| G1PAE6 | weaned pig | <i>bla</i> _{CTX-M-1} | neg |
| G1PAE7 | weaned pig | <i>bla</i> _{CTX-M-1} | <i>bla</i> _{TEM-1} |
| G1PAE8 | weaned pig | <i>bla</i> _{CTX-M-1} | <i>bla</i> _{TEM-1} |
| G1PAE9 | weaned pig | <i>bla</i> _{CTX-M-1} | neg |
| G1PHE1 | farm environment | <i>bla</i> _{CTX-M-15} | neg |
| G1PHE2 | farm environment | <i>bla</i> _{CTX-M-1} | neg |
| G1SAE2 | sow | <i>bla</i> _{CTX-M-1} | neg |
| G1SAE4 | sow | <i>bla</i> _{CTX-M-1} | neg |
| G1SAE7 | sow | <i>bla</i> _{CTX-M-1} | neg |
| G1SAE8 | sow | <i>bla</i> _{CTX-M-1} | neg |
| G1SAE10 | sow | <i>bla</i> _{CTX-M-1} | neg |
| P1PAE2 | weaned | <i>bla</i> _{CTX-M-14} | <i>bla</i> _{TEM-1} |

| | | | |
|---------|------------------|---------------------------------|-----------------------------|
| P1PAE3 | weaned | <i>bla</i> _{CTX-M-14} | <i>bla</i> _{TEM-1} |
| P1PAE4 | weaned | <i>bla</i> _{CTX-M-14} | <i>bla</i> _{TEM-1} |
| P1PAE6 | weaned pig | <i>bla</i> _{CTX-M-14} | <i>bla</i> _{TEM-1} |
| P1PAE7 | weaned pig | <i>bla</i> _{CTX-M-14} | <i>bla</i> _{TEM-1} |
| P1PAE8 | weaned pig | <i>bla</i> _{CTX-M-14} | <i>bla</i> _{TEM-1} |
| P1PAE9 | weaned pig | <i>bla</i> _{CTX-M-14} | <i>bla</i> _{TEM-1} |
| P1PAE10 | weaned pig | <i>bla</i> _{CTX-M-14} | <i>bla</i> _{TEM-1} |
| P1FAE7 | finishing pig | <i>bla</i> _{CTX-M-1} | <i>bla</i> _{TEM-1} |
| P1FAE3 | finishing pig | <i>bla</i> _{CTX-M-14} | neg |
| P1FAE1 | finishing pig | <i>bla</i> _{CTX-M-14} | <i>bla</i> _{TEM-1} |
| S1FHE2 | farm environment | <i>bla</i> _{CTX-M-14*} | neg |
| T1SAE6 | sow | <i>bla</i> _{CTX-M-1} | neg |
| T1SAE7 | sow | <i>bla</i> _{CTX-M-1} | <i>bla</i> _{TEM-1} |
| T1SAE8 | sow | neg | <i>bla</i> _{TEM-1} |
| T1SAE9 | sow | <i>bla</i> _{CTX-M-1} | neg |
| T1SAE10 | sow | <i>bla</i> _{CTX-M-1} | neg |

Supplementary Table S2: AMR associated genes, with relative function and frequency, detected by WGS in ESBL *E. coli* sampled from healthy pigs and farm environment, northern Italy.

| AMR gene | Function | Number of strains with the gene and frequency (%) |
|-------------------------------|---|---|
| <i>aac(6')-Ib-cr</i> | Fluoroquinolones and aminoglycosides resistance | 10; 33.3% |
| <i>aac(6')-Ib3</i> | Amikacin, tobramycin resistance | 10; 33.3% |
| <i>aph(3'')-Ib</i> | Aminoglycoside resistance | 6; 20% |
| <i>aph(6)-Id</i> | Aminoglycoside resistance | 1; 3.3% |
| <i>aph(6')-Id</i> | Aminoglycoside resistance | 4; 13.3% |
| <i>aph(4)-Ia</i> | Aminoglycoside resistance | 1; 3.3% |
| <i>aadA1</i> | Aminoglycoside resistance | 23; 76.7% |
| <i>aadA2</i> | Aminoglycoside resistance | 5; 16.7% |
| <i>aadA2b</i> | Aminoglycoside resistance | 10; 33.3% |
| <i>aadA5</i> | Aminoglycoside resistance | 8; 26.7% |
| <i>bla_{CTX-M-1}</i> | Beta-lactam resistance | 16; 53.3% |
| <i>bla_{CTX-M-14}</i> | Beta-lactam resistance | 11; 36.7% |
| <i>bla_{CTX-M-15}</i> | Beta-lactam resistance | 1; 3.3% |
| <i>bla_{TEM-1A}</i> | Beta-lactam resistance | 2; 6.7% |
| <i>bla_{TEM-1B}</i> | Beta-lactam resistance | 12; 40% |
| <i>bla_{TEM-1C}</i> | Beta-lactam resistance | 3; 10% |

| | | |
|----------------|--|-----------|
| <i>catA1</i> | Chloramphenicol resistance | 8; 26.7% |
| <i>cmlA1</i> | Phenicol resistance | 28; 93.3% |
| <i>dfrA12</i> | Trimethoprim resistance | 8; 26.7% |
| <i>dfrA17</i> | Trimethoprim resistance | 8; 26.7% |
| <i>dfrA36</i> | Trimethoprim resistance | 1; 3.3% |
| <i>erm(B)</i> | Macrolide resistance | 1; 3.3% |
| <i>floR</i> | Phenicol resistance | 1; 3.3% |
| <i>mdf(a)</i> | Multiple resistance to benzalkonium chloride, daunomycin, rhodamine | 30; 100% |
| <i>mph(A)</i> | Macrolide resistance | 16; 53.3% |
| <i>mph(B)</i> | Macrolide resistance | 2; 6.7% |
| <i>qnrS1</i> | Quinolone resistance | 11; 36.7% |
| <i>sitABCD</i> | Peroxide hydrogen resistance | 16; 53.3% |
| <i>sul1</i> | Sulphonamide resistance | 2; 6.7% |
| <i>sul2</i> | Sulphonamide resistance | 10; 33.3% |
| <i>sul3</i> | Sulphonamide resistance | 17; 56.7% |
| <i>tet(A)</i> | Tetracycline resistance | 19; 63.3% |
| <i>tet(B)</i> | Tetracycline resistance | 6; 20% |

Supplementary Table S3: virulence genes, with relative function and frequency, detected by WGS in ESBL *E. coli* sampled from healthy pigs and farm environment, northern Italy.

| Virulence gene | function | Number of strains with the gene and frequency (%) |
|----------------|--|---|
| <i>astA</i> , | EAST-1 heat-stable toxin | 6; 20% |
| <i>cea</i> | colicin E1 | 11; 36.7% |
| <i>celb</i> , | Endonuclease colicin E2 | 7; 23.3% |
| <i>chua</i> | Outer membrane hemin receptor | 4; 13.3% |
| <i>cia</i> | Colicin ia | 10; 33.3% |
| <i>cib</i> | Colicin ib | 5; 16.7% |
| <i>cma</i> | Colicin M | 1; 3.3% |
| <i>cvaC</i> , | Microcin C | 14; 46.7% |
| <i>eilA</i> | Salmonella HilA homolog | 3; 10% |
| <i>etsC</i> | Putative type I secretion outer membrane protein | 14; 46.7% |
| <i>fyuA</i> | Siderophore receptor | 2; 6.7% |
| <i>gad</i> | Glutamate decarboxylase | 6; 20% |
| <i>hlyF</i> | Hemolysin F | 15; 50% |
| <i>hra</i> | Heat-resistant agglutinin | 4; 13.3% |
| <i>ireA</i> | Siderophore receptor | 1; 3.3% |
| <i>iroN</i> , | Enterobactin siderophore receptor protein | 14; 46.7% |
| <i>lrp2</i> | High molecular weight protein 2 non-ribosomal peptide synthetase | 2; 6.7% |
| <i>iss</i> | Increased serum survival | 16; 53.3% |
| <i>iucC</i> | Aerobactin synthetase | 15; 50% |

| | | |
|------------------|--|-----------|
| <i>iutA</i> , | Ferric aerobactin receptor | 15; 50% |
| <i>katP</i> | Plasmid-encoded catalase peroxidase | 1; 3.3% |
| <i>kpsE</i> , | Capsule polysaccharide export inner-membrane protein | 5; 16.7% |
| <i>kpsMII</i> , | Polysialic acid transport protein; Group 2 capsule | 5; 16.7% |
| <i>kpsMII_K5</i> | Polysialic acid transport protein; Group 2 capsule | 1; 3.3% |
| <i>lpfA</i> | Long polar fimbriae | 21; 70% |
| <i>ltcA</i> | Heat-labile enterotoxin A subunit | 2; 6.7% |
| <i>mchF</i> , | ABC transporter protein MchF | 15; 50% |
| <i>neuC</i> | polysialic acid capsule biosynthetic protein | 1; 3.3% |
| <i>ompT</i> | Outer membrane protease (protein protease 7) | 20; 66.7% |
| <i>papC</i> , | Outer membrane usher P fimbriae | 2; 6.7% |
| <i>sitA</i> , | Iron transport protein | 16; 53.3% |
| <i>stb</i> | Heat-stable enterotoxin II | 3; 10% |
| <i>terC</i> , | Tellurium ion resistance protein | 30; 100% |
| <i>traT</i> | Outer membrane protein complement resistance | 21; 70% |
| <i>tsh</i> | Temperature-sensitive hemagglutinin | 5; 16.7% |
| <i>vat</i> | Vacuolating autotransporter toxin | 1; 3.3% |

Supplementary Table S4: potential human pathogenicity, expressed as percentage, of ESBL *E. coli* sampled from healthy pigs and farm environment, northern Italy. Results were obtained through PathogenFinder 1.1 (<https://cge.cbs.dtu.dk/services/>).

| Strains | Probability of human pathogenicity |
|---------|------------------------------------|
| G1PAE2 | 88% |
| G1PAE3 | 88% |
| G1PAE4 | 88% |
| G1PAE7 | 88% |
| G1PAE8 | 89% |
| G1PAE9 | 87% |
| G1PHE1 | 84% |
| G1PHE2 | 93% |
| G1SAE2 | 94% |
| G1SAE4 | 89% |
| G1SAE8 | 89% |
| G1SAE7 | 95% |
| G1SAE10 | 89% |
| P1FAE1 | 93% |
| P1FAE3 | 93% |
| P1FAE7 | 92% |
| P1PAE2 | 93% |

| | |
|----------------|-----|
| P1PAE3 | 89% |
| P1PAE4 | 93% |
| P1PAE6 | 89% |
| P1PAE7 | 89% |
| P1PAE8 | 89% |
| P1PAE9 | 89% |
| P1PAE10 | 93% |
| S1FHE2 | 88% |
| T1SAE6 | 83% |
| T1SAE7 | 88% |
| T1SAE8 | 87% |
| T1SAE9 | 88% |
| T1SAE10 | 83% |

Supplementary Table S5: WGS quality parameters of all analysed *E. coli* strains.

| Strains | Contigs | Reads/length reads | Coverage | N50 | length | GC% |
|----------------|----------------|---------------------------|-----------------|------------|---------------|------------|
| G1PAE2 | 70 | 1006488/ 224 | 46x | 240995 | 4908608 | 50.45 |
| G1PAE3 | 94 | 541164/ 234 | 26x | 157912 | 4937072 | 50.39 |

| | | | | | | |
|---------|-----|-----------------|-----|--------|---------|-------|
| G1PAE4 | 63 | 1122266/ 233 | 53x | 240584 | 4907891 | 50.45 |
| G1PAE7 | 151 | 896196/ 220 | 37x | 97282 | 5291539 | 50.47 |
| G1PAE8 | 66 | 911856/ 232 | 42x | 209216 | 5057771 | 50.71 |
| G1PAE9 | 51 | 1198842/ 236 | 58x | 208729 | 4852108 | 50.65 |
| G1PHE1 | 121 | 787752/ 235 | 40x | 73509 | 4612127 | 50.61 |
| G1PHE2 | 86 | 708166/ 229 | 32x | 179714 | 5132633 | 50.62 |
| G1SAE2 | 161 | 533048/ 235 | 26x | 71036 | 4862726 | 50.55 |
| G1SAE4 | 99 | 1367040/ 224 | 64x | 95864 | 4802459 | 50.65 |
| G1SAE8 | 100 | 615278/ 224 | 29x | 91912 | 4801399 | 50.66 |
| G1SAE10 | 101 | 1126712/ 223 | 52x | 95864 | 4802832 | 50.64 |

| | | | | | | |
|--------|-----|-----------------|-----|--------|---------|-------|
| P1FAE1 | 72 | 1728010/ 232 | 78x | 209689 | 5110356 | 50.61 |
| P1FAE3 | 145 | 1168964/ 231 | 52x | 111436 | 5175856 | 50.75 |
| P1FAE7 | 157 | 2051676/ 229 | 97x | 76744 | 4837638 | 50.68 |
| P1PAE2 | 295 | 432122/ 230 | 20x | 41072 | 5078038 | 50.52 |
| P1PAE3 | 73 | 1084192/ 219 | 45x | 297184 | 5267640 | 50.42 |
| P1PAE4 | 119 | 563930/ 229 | 25x | 145436 | 5124857 | 50.51 |
| P1PAE6 | 97 | 1196434/ 221 | 52x | 179311 | 5124981 | 50.52 |
| P1PAE7 | 167 | 530858/ 226 | 23x | 70982 | 5106936 | 50.52 |
| P1PAE8 | 105 | 1093036/ 218 | 47x | 179593 | 5121840 | 50.52 |
| P1PAE9 | 98 | 1145512/ 225 | 50x | 177776 | 5125014 | 50.52 |

| | | | | | | |
|---------|-----|-----------------|------|--------|---------|-------|
| P1PAE10 | 92 | 2444398/ 219 | 106x | 189765 | 5026802 | 50.57 |
| S1FHE2 | 127 | 967314/ 219 | 40x | 176978 | 5316621 | 50.50 |
| T1SAE6 | 101 | 845024/ 205 | 36x | 105200 | 4777837 | 50.77 |
| T1SAE7 | 95 | 2079994/ 219 | 94x | 136287 | 4841785 | 50.68 |
| T1SAE8 | 116 | 1029662/ 224 | 51x | 75014 | 4543562 | 50.81 |
| T1SAE9 | 82 | 1074586/ 223 | 50x | 127839 | 4756721 | 50.60 |
| T1SAE10 | 99 | 826014/ 224 | 38x | 111806 | 4853313 | 50.90 |

Supplementary table S6: identification of virulence and AMR genes on plasmids located on specific contigs of G1PAE2 genome. To confirm the presence of virulence and/or AMR genes on particular plasmids, tools (PlasmidFinder, ResFinder and VirulenceFinder) from Center for Genomic Epidemiology (<https://cge.cbs.dtu.dk/services/>) were used. NS, no significant hits.

| Strain | Contig | PlasmidFinder | VirulenceFinder | ResFinder |
|--------|--------|---|-----------------|-----------|
| G1PAE2 | 34 | IncFIA 99.74 388 / 388 G1PAE2_S18_L001_R1_001_(paired_trimmed_pairs)_cont ig_34 6022..6408 - AP001918 | NS | NS |

| | | | | |
|--|----|--|---|--|
| | 31 | IncFIB(AP001918) 99.56 682 / 682 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_31 7274..7955 - AP001918 | hlyF 100 1110 / 1110 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_31 3116..4225 Hemolysin F UGFJ01000001 ompT 99.79 954 / 954 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_31 1730..2683 Outer membrane protease (protein protease 7) MG692627 | NS |
| | 36 | IncFIC(FII) 95.79 499 / 499 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_36 24152..24648 - AP001918 | cia 94.07 253 / 264 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_36 28783..29035 Colicin ia UGDH01000002 traT 100 831 / 831 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_36 11870..12700 Outer membrane protein complement resistance NC_011964 | NS |
| | 9 | IncI1-I(Alpha) 99.3 142 / 142 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_9 9349..9490 - AP005147 | NS | NS |
| | 43 | IncX1 99.2 377 / 377 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_43 6528..6904 - JN935898 | NS | NS |
| | 46 | NS | NS | <p>aadA1 100 792/792 100 1..792 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_46 10820..11611 Aminoglycoside resistance JQ414041 </p> <p>aadA2b 99.8718 780/780 100 1..780 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_46 8427..9206 Aminoglycoside resistance D43625</p> <p>sul3 100 792/792 100 1..792 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_46 13293..14084 Sulphonamide resistance AJ459418</p> |

| | | | | |
|--|----|----|----|---|
| | | | | <p>blaCTX-M-1 100 876/876 100 1..876 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_46 18284..19159 Beta-lactam resistance DQ915955</p> <p>qacL 92.2156 501/533 93.9962 1..501 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_46 11681..12181 Disinfectant resistance NG_048048</p> <p>catA1 99.8485 660/660 100 1..660 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_46 119..778 Phenicol resistance V00622 </p> <p>cmlA1 99.9206 1260/1260 100 1..1260 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_46 9468..10727 Phenicol resistance M64556</p> |
| | 55 | NS | NS | <p>tet(A) 100 1200/1200 100 1..1200 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_55 2349..3548 Tetracycline resistance AJ517790</p> <p>blaTEM-1C 100 861/861 100 1..861 G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_55 4041..4901 Beta-lactam resistance Alternate name; RblaTEM-1 FJ560503</p> |

Supplementary table S7: comparison of plasmid-positive contigs found in G1PAE2 *E. coli* strain with sequences recorded in BLAST[®] database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The best match is reported in the last column on the right

| Strain | Contig | Length | Best BLAST hit |
|--------|--------|--------|---|
| G1PAE2 | 9 | 27044 | plasmid G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_9 KJ484639.1 99.945 25634 6 8 1 25626 63878 89511 0.0 47253 |
| | 31 | 9806 | plasmid G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_31 MG649031.1 99.959 9806 4 0 1 9806 10030 225 0.0 18087 |
| | 34 | 13565 | plasmid G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_34 CP056956.1 99.978 13565 3 0 1 13565 131729 118165 0.0 25034 |
| | 36 | 30201 | plasmid G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_36 CP056956.1 99.985 13637 2 0 16565 30201 78444 64808 0.0 25172 |
| | 43 | 44395 | plasmid G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_43 CP062857.1 99.955 35762 16 0 8634 44395 44395 8634 0.0 65952 |
| | 46 | 22534 | plasmid G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_46 CP026933.2 100.000 15917 0 0 20 15936 176072 160156 0.0 29394 |
| | 55 | 5785 | plasmid G1PAE2_S18_L001_R1_001_(paired,_trimmed_pairs)_contig_55 OW848878.1 100.000 4445 0 0 1341 5785 13217 8773 0.0 8209 |

