

Supplementary Material

# Antimicrobial Resistance and Virulence Factors Assessment in *Escherichia coli* Isolated from Swine in Italy from 2017 to 2021

**Table S1.** Different combination of adhesins and toxins of 457 ETEC isolates, N: number and (%): percentage. STa: heat-stable toxin a; STb: heat-stable toxin b; LT: heat-labile toxin; Stx2e: Shiga toxin 2e.

Adhesins		Toxins																		
		STa		STb		LT		STa, STb		STa, LT		STa, Stx2e		STb, LT		STa, STb, LT		STa, STb, Stx2e		STa, STb, LT, Stx2e
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
F18	20	4.4	6	1.3	1	0.2	136	29.8	4	0.9	7	1.5	1	0.2	30	6.6	22	4.8	3	0.7
F4	7	1.5	12	2.6	10	2.2	40	8.8	8	1.8	-	-	32	7	35	7.7	-	-	-	-
F5	15	3.3	2	0.4	1	0.2	2	0.4	1	0.2	-	-	-	-	1	0.2	-	-	-	-
F4 F18	3	0.7	1	0.2	-	-	14	3.1	-	-	-	-	-	-	3	0.7	-	-	-	-
F41	8	1.8	6	1.3	-	-	5	1.1	-	-	-	-	-	-	-	-	-	-	-	-
F5 F41	14	3.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F6	1	0.2	2	0.4	1	0.2	1	0.2	-	-	-	-	-	-	-	-	-	-	-	-
F4 F6	-	-	-	-	-	-	-	-	-	-	-	-	1	0.2	-	-	-	-	-	-
F5 F6	-	-	-	-	-	-	1	0.2	-	-	-	-	-	-	-	-	-	-	-	-

**Table S2.** Percentage (%) of resistance to selected antibiotics from 2017 to 2021 and statistical analysis of observed variations (*r*) listed for all the 826 *E. coli* isolated from swine pathologic samples, and for F18+ and F4+ isolates. Nalidixic acid (NA), amoxicillin and clavulanic acid (AMC), ampicillin (AMP), cefazolin (CZ), enrofloxacin (ENR), florfenicol (FFC); gentamicin (GEN), kanamycin (KAN), tetracycline (TET), trimethoprim + sulfamethoxazole (SXT).

Antibiotics	% of resistant strains					Statistical analysis	
Year of isolation	2017	2018	2019	2020	2021	r	p
NA	84.1	73.6	68.7	69.3	74.2	-0.62	>0.05
AMC	61.7	52.8	63.1	68.9	74.2	0.88	>0.05
AMP	93.5	94.4	95.9	96.1	99.2	0.96	<0.05*
CZ	73.8	83.2	78.3	80.5	79.2	0.37	>0.05
ENR	64.5	64.8	54.4	51	60.8	-0.54	>0.05
FFC	61.7	65.6	63.1	62.3	69.2	0.60	>0.05
GEN	58.9	55.2	64.5	61.9	63.3	0.66	>0.05
KAN	48.6	54.4	63.1	63	59.2	0.76	>0.05
TET	92.5	96	92.6	85.2	85	-0.83	>0.05
SXT	70.1	73.6	78.8	75.1	72.5	0.31	>0.05
N° tested isolates	107	125	217	257	120		

Antibiotics -	% of resistances of F18+ strains					Statistical analysis	
Year of isolation	2017	2018	2019	2020	2021	r	p
NA	90	79.4	68.4	77.6	91.7	0.03	>0.05
AMC	50	38.2	59.2	65.8	79.2	0.87	>0.05
AMP	95	97.1	96.1	93.4	95.8	-0.23	>0.05
CZ	65	91.2	73.7	75	83.3	0.32	>0.05
ENR	55	79.4	42.1	48.7	54.2	-0.36	>0.05
FFC	70	79.4	80.3	81.6	95.8	0.92	<0.05*
GEN	70	79.4	80.3	78.9	91.7	0.88	<0.05*

KAN	50	50	71.1	69.7	91.7	0.94	<0.05*
TET	95	100	96.1	90.8	87.5	-0.79	>0.05
SXT	65	88.2	88.2	85.5	83.3	0.55	>0.05
N° tested isolates	20	34	76	76	24		
Antibiotics -		% of resistances of F4+ strains				Statistical analysis	
Year of isolation	2017	2018	2019	2020	2021	r	p
NA	88	85.2	67.5	73	93.3	-0.02	>0.05
AMC	60	55.6	60	59.5	73.3	0.71	>0.05
AMP	96	92.6	95	94.6	100	0.58	>0.05
CZ	88	81.5	77.5	83.8	66.7	-0.79	>0.05
ENR	72	66.7	57.5	59.5	73.3	-0.10	>0.05
FFC	60	66.7	55	62.2	73.3	0.51	>0.05
GEN	60	51.9	47.5	64.9	66.7	0.50	>0.05
KAN	48	55.6	50	59.5	60	0.81	>0.05
TET	92	96.3	82.5	75.7	86.7	-0.61	>0.05
SXT	68	51.9	60	59.5	73.3	0.35	>0.05
N° tested isolates	25	27	40	37	15		

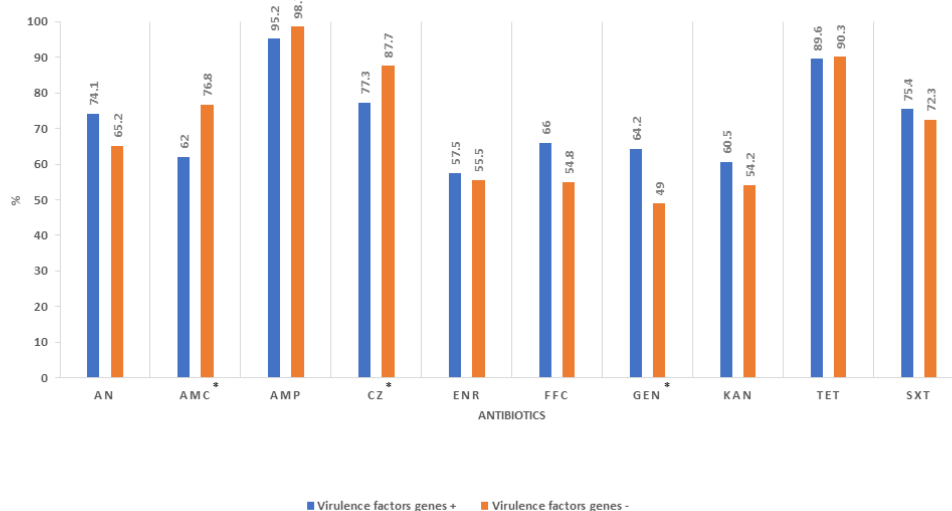
\*the trend was considered statistically significant for  $p < 0.05$

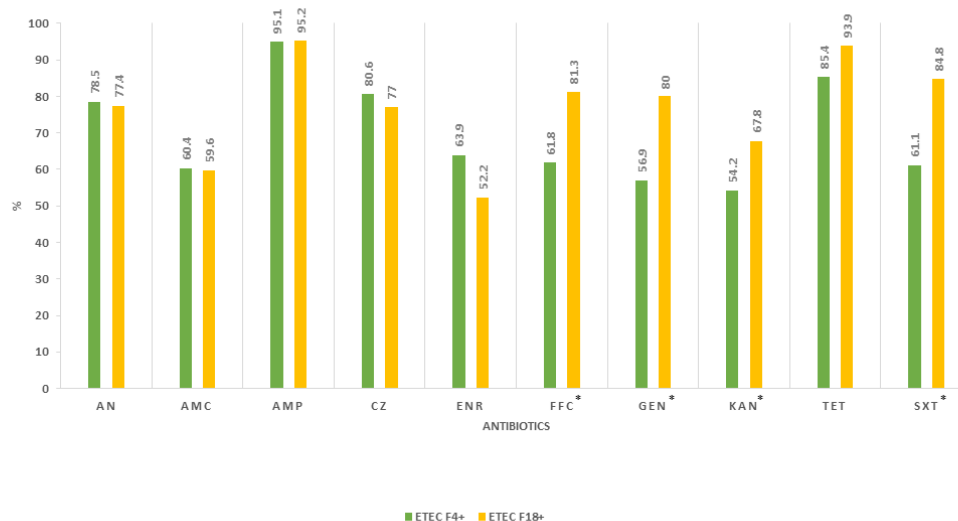
**Table S3.** Percentages (%) of *E. coli* F4+ isolates simultaneously resistant to n° antibiotics (from 1 to 10) and to  $\geq 6$  antibiotics (multiresistant) from 2017 to 2021. Nalidixic acid (NA), amoxicillin and clavulanic acid (AMC), ampicillin (AMP), cefazolin (CZ), enrofloxacin (ENR), florfenicol (FFC); gentamicin (GEN), kanamycin (KAN), tetracycline (TET) trimethoprim + sulfamethoxazole (SXT). The trend was considered statistically significant for  $p < 0.05$ .

% of resistances to n° antibiotics of F4+ strains								
n° antibiotics	Total	Year of isolation					Statistical analysis	
		2017	2018	2019	2020	2021	r	p
0	0	0	0	0	0	0	-	-
1	2.1	0	3.7	2.5	2.7	0	-0.09	>0.05
2	2.1	0	0	2.5	5.4	0	0.36	>0.05
3	7.6	4	11.1	7.5	10.8	0	-0.28	>0.05
4	6.3	8	3.7	7.5	5.4	6.7	-0.09	>0.05
5	6.3	12	3.7	7.5	2.7	6.7	-0.50	>0.05
6	13.2	16	14.8	15	8.1	13.3	-0.61	>0.05
7	14.6	8	11.1	20	10.8	26.7	0.75	>0.05
8	16.7	8	14.8	22.5	21.6	6.7	0.09	>0.05
9	14.6	28	22.2	5	10.8	13.3	-0.70	>0.05
10	16.7	16	14.8	10	21.6	26.7	0.69	>0.05
>6	75.7	76	77.8	72.5	73	86.7	0.46	>0.05
N° tested isolates	144	25	27	40	37	15		

**Table S4.** Virulence factor genes specific primers [22]. Fimbriae (F), heat-labile toxin (LT); heat-stable toxin a (STa); heat-stable toxin b (STb); Shiga toxin (Stx).

Virulence factor	F 5'-3'	R 5'-3'
F4	GTTGGTACAGGTCTTAATGG	GAATCTGTCCGAGAATATCA
F5	AATACTTGTTTCAGGGAGAAA	AACTTTGTGGTTAACTTCCT
F6	AAGTTACTGCCAGTCTATGC	GTAACCTCCACCGTTTGTATC
F18	TGGTAACGTATCAGCAACTA	ACTTACAGTGCTATTTCGACG
F41	AGTATCTGGTTCAGTGATGG	CCACTATAAGAGGTTGAAGC
LTb subunit	GGCGTTACTATCCTCTCTAT	TGGTCTCGGTCAGATATGT
STaP	CAACTGAATCACTTGACTCTT	TTAATAACATCCAGCACAGG
STb	TGCCTATGCATCTACACAAT	CTCCAGCAGTACCATCTCTA
Stx2e	AATAGTATACGGACAGCGAT	TCTGACATTCTGGTTGACTC

**Figure S1.** Percentage of resistance to the tested antibiotics of virulence factor genes positive and negative *Escherichia coli*. Nalidixic acid (NA), amoxicillin and clavulanic acid (AMC), ampicillin (AMP), cefazolin (CZ), enrofloxacin (ENR), florfenicol (FFC); gentamicin (GEN), kanamycin (KAN), tetracycline (TET) trimethoprim + sulfamethoxazole (SXT). \* Percentage of isolates resistant to antimicrobials with  $p < 0.01$ .



**Figure S2.** Percentage of resistance to the tested antibiotics of Enterotoxigenic *Escherichia coli* (ETEC) positive to Fimbrial factor (F) 4 or 18. Nalidixic acid (NA), amoxicillin and clavulanic acid (AMC), ampicillin (AMP), cefazolin (CZ), enrofloxacin (ENR), florfenicol (FFC); gentamicin (GEN), kanamycin (KAN), tetracycline (TET) trimethoprim + sulfamethoxazole (SXT). \* Percentage of isolates resistant to antimicrobials with  $p < 0.01$ .