Supplementary data

Methods

The list of antimicrobial compounds that were tested was through the disk diffusion assay included: amoxicillin, amoxicillin-clavulanic acid, ticarcillin, ticarcillin-clavulanic acid, piperacillin, piperacillin-tazobactam, temocillin, cefoxitin, cefixime, ceftazidime, cefotaxime, cefepim,aztreonam ertapenem, meropenem, imipenem, amikacin, gentamicin, tobramycin, netilmicin, cotrimoxazole, norfloxacin, ofloxacin, levofloxacin, ciprofloxacin, fosfomycin, tigecycline.

Whole-genome sequences obtained from Illumina NextSeq 2x150 bp were analysed with an in-house bioinformatic pipeline named PETANC for "Plasmid-Exploration Typing Assembly N'Contig-ordering", which we describe below.

MLST (Warwick and Pasteur Institute schemes) and serotype are determined using SRST2 0.2.0 with standard parameters.[9] Previously unknown Warwick STs are determined on EnteroBase website (https://enterobase.warwick.ac.uk/). Genomes are assembled with SPAdes 3.10.0 with "careful" option. The distances between a set of 313 reference *E. coli* complete circularised genomes (Table S1) and the chromosome sequences of the studied isolates are estimated using Mash 1.1.1.[50] The 5 closest reference genomes for each studied isolate are determined with an in-house biopython script.[51] These are used as reference for Ragout software, with option "solid-scaffolds", to determine the order of the contigs and therefore enhance the assembly quality.[7] Subsequently, the phylogroup of each isolate is determined with the ClermonTyper.[8] Then, Abricate is used (https://github.com/tseemann/abricate) to determine i) the resistome with the Resfinder database using minimum identity (minID) of 95% and minimum coverage (minCOV) of 90%, ii) the virulome (minID 90%, minCOV 90%) with a custom database mixing Virulencefinder,

VFDB, and specific genes from extra-intestinal *E. coli* (Table S2), iii) the plasmid type with PlasmidFinder database (minID 90%, mincov 90%).[10,11,52,53] InPEC and ExPEC virulence scores were computed based on presence/absence of InPEC and ExPEC virulence genes as previously described.[34,54] Subsequently, chromosome and plasmid sequences are predicted with PlaScope and resistome and virulome are reassessed in the same way on the predictions to determine gene's location.[12,55]

Table S1. Accession number of 313 *E. coli* reference genomes for Ragout ordering

NC_000913	NC_013364	NZ_AP014857	NZ_CP009166	NZ_CP010170	NZ_CP010344	NZ_CP012127	NZ_CP014316	NZ_CP015912	NZ_CP018237	NZ_CP019629
NC_002695	NC_013654	NZ_CP005930	NZ_CP009273	NZ_CP010171	NZ_CP010371	NZ_CP012625	NZ_CP014348	NZ_CP015995	NZ_CP018239	NZ_CP019777
NC_004431	NC_013941	NZ_CP005998	NZ_CP009644	NZ_CP010172	NZ_CP010438	NZ_CP012631	NZ_CP014488	NZ_CP016007	NZ_CP018241	NZ_CP019778
NC_007779	NC_016902	NZ_CP006027	NZ_CP009685	NZ_CP010176	NZ_CP010439	NZ_CP012633	NZ_CP014492	NZ_CP016018	NZ_CP018243	NZ_CP019903
NC_007946	NC_017625	NZ_CP006262	NZ_CP009789	NZ_CP010177	NZ_CP010440	NZ_CP012635	NZ_CP014495	NZ_CP016182	NZ_CP018245	NZ_CP019944
NC_008253	NC_017626	NZ_CP006632	NZ_CP009859	NZ_CP010178	NZ_CP010441	NZ_CP012693	NZ_CP014497	NZ_CP016358	NZ_CP018247	NZ_CP020048
NC_008563	NC_017628	NZ_CP006636	NZ_CP010116	NZ_CP010180	NZ_CP010442	NZ_CP012802	NZ_CP014522	NZ_CP016404	NZ_CP018250	NZ_CP020055
NC_009800	NC_017631	NZ_CP007025	NZ_CP010117	NZ_CP010183	NZ_CP010443	NZ_CP012868	NZ_CP014583	NZ_CP016497	NZ_CP018252	NZ_CP020058
NC_009801	NC_017632	NZ_CP007133	NZ_CP010119	NZ_CP010186	NZ_CP010444	NZ_CP012869	NZ_CP014641	NZ_CP016546	NZ_CP018770	NZ_CP020092
NC_010468	NC_017633	NZ_CP007136	NZ_CP010121	NZ_CP010191	NZ_CP010445	NZ_CP012870	NZ_CP014642	NZ_CP016625	NZ_CP018801	NZ_CP020106
NC_010473	NC_017634	NZ_CP007149	NZ_CP010122	NZ_CP010196	NZ_CP010585	NZ_CP013025	NZ_CP014667	NZ_CP016628	NZ_CP018948	NZ_CP020107
NC_010498	NC_017635	NZ_CP007265	NZ_CP010125	NZ_CP010200	NZ_CP010816	NZ_CP013029	NZ_CP014670	NZ_CP017100	NZ_CP018953	NZ_CP020116
NC_011353	NC_017638	NZ_CP007390	NZ_CP010129	NZ_CP010206	NZ_CP010876	NZ_CP013031	NZ_CP015020	NZ_CP017249	NZ_CP018957	NZ_CP020368
NC_011415	NC_017641	NZ_CP007391	NZ_CP010132	NZ_CP010213	NZ_CP011018	NZ_CP013112	NZ_CP015023	NZ_CP017251	NZ_CP018962	NZ_CP020543
NC_011601	NC_017646	NZ_CP007392	NZ_CP010133	NZ_CP010219	NZ_CP011061	NZ_CP013190	NZ_CP015069	NZ_CP017434	NZ_CP018965	NZ_HF572917
NC_011740	NC_017651	NZ_CP007393	NZ_CP010134	NZ_CP010221	NZ_CP011113	NZ_CP013253	NZ_CP015074	NZ_CP017436	NZ_CP018970	NZ_HG738867
NC_011741	NC_017652	NZ_CP007394	NZ_CP010137	NZ_CP010226	NZ_CP011124	NZ_CP013483	NZ_CP015076	NZ_CP017438	NZ_CP018976	NZ_HG941718
NC_011742	NC_017656	NZ_CP007442	NZ_CP010140	NZ_CP010228	NZ_CP011134	NZ_CP013658	NZ_CP015138	NZ_CP017440	NZ_CP018979	NZ_LM993812
NC_011748	NC_017660	NZ_CP007491	NZ_CP010143	NZ_CP010229	NZ_CP011320	NZ_CP013662	NZ_CP015159	NZ_CP017442	NZ_CP018983	NZ_LM995446
NC_011750	NC_017663	NZ_CP007592	NZ_CP010145	NZ_CP010230	NZ_CP011321	NZ_CP013663	NZ_CP015228	NZ_CP017444	NZ_CP018991	NZ_LN832404
NC_011993	NC_017664	NZ_CP007594	NZ_CP010148	NZ_CP010231	NZ_CP011324	NZ_CP013831	NZ_CP015229	NZ_CP017446	NZ_CP018995	NZ_LT601384
NC_012759	NC_017906	NZ_CP007799	NZ_CP010150	NZ_CP010235	NZ_CP011331	NZ_CP013835	NZ_CP015240	NZ_CP017631	NZ_CP019000	NZ_LT615377
NC_012892	NC_018650	NZ_CP008697	NZ_CP010151	NZ_CP010236	NZ_CP011342	NZ_CP014197	NZ_CP015241	NZ_CP017669	NZ_CP019005	NZ_LT615378
NC_012947	NC_018658	NZ_CP008801	NZ_CP010152	NZ_CP010237	NZ_CP011343	NZ_CP014225	NZ_CP015831	NZ_CP017844	NZ_CP019008	
NC_012967	NC_018661	NZ_CP008805	NZ_CP010157	NZ_CP010238	NZ_CP011416	NZ_CP014268	NZ_CP015832	NZ_CP018103	NZ_CP019012	
NC_012971	NC_020163	NZ_CP008957	NZ_CP010160	NZ_CP010240	NZ_CP011495	NZ_CP014269	NZ_CP015834	NZ_CP018109	NZ_CP019015	
NC_013008	NC_020518	NZ_CP009072	NZ_CP010163	NZ_CP010242	NZ_CP011938	NZ_CP014270	NZ_CP015842	NZ_CP018115	NZ_CP019020	
NC_013353	NC_022364	NZ_CP009104	NZ_CP010167	NZ_CP010304	NZ_CP012125	NZ_CP014272	NZ_CP015843	NZ_CP018121	NZ_CP019029	
NC_013361	NC_022648	NZ_CP009106	NZ_CP010169	NZ_CP010315	NZ_CP012126	NZ_CP014314	NZ_CP015846	NZ_CP018206	NZ_CP019213	

Table S2. Specific extra-intestinal *E. coli* virulence genes with their name and accession number

Gene name	**Accession number**
cea	U15633
Chromosomal_*ompT*	CP025268:583729-584682
clbQ	AE014075:2265084-2265806
cvaC	CP019268:43764-44075
Episomal_*ompT*	CP019282:79325-80278
etsC	JX077110:124931-126301
fyuA	CP009836:3377794-3379815
hek	CP000243:4785401-4786156
hlyF	CU928146:131210-132331
irp2	CP006806:2552888-2558995
kpsE	CU928164:3584271-3585419
malX	CP000247:1618614-1620206
mcbA	M24253:408-614
neuC	M84026
papGI	X61239:8679-9686
papGII	AE014075:3429593-3430603
papGIII	CP000243:4789101-4790108
terC	AE005174:1102902-1103942
tia	CU928161:3229672-3230427
traT	J01769.1:403-1134
usp	CP000247:123276-125057
hra	U07174.1:83-826

Table S3. The antimicrobial susceptibility, the resistome and the genetic support of the *mcr-1* gene of the plasmid mediated colistin resistant *E. coli* strains

Strain ID	**Colistin MIC (mg/L)**	**Antimicrobial resistance***	**Resistome****		*mcr* genetic support	
			Acquired gene	Chromosomal mutation	Incompatibility group	Associated resistance
370D	4	AMP, LLFQ, SXT	*strB, strA, aadA2, aph(3')-Ia, oqxA, oqxB, dfrA12, tet(B), tet(M), sul2, sul3, cmlA1*	*gyr*A (S83L)	IncX4	none
436A	4	SXT	*strB, strA, aadA5, qnrS1, dfrA17, tet(A), sul2*	-	IncX4	none
635A	4	AMP, GTN, SXT	*blaTEM-1b, strB, strA, aadA2, aph(3')-Ic, aac(3)-IId, qnrS1, dfrA12, tet(A), tet(M), sul1, sul3, catA2, mph(A)*	-	IncX4	none
925B	8	HLFQ	-	*gyr*A (S83L, D87N), *par*C (S80I)	IncI2	none
933A	8	AMP, HLFQ, SXT	*blaTEM-1b, strB, strA, dfrA14, tet(A), sul2, floR, catA1*	*gyr*A (D87G, S83L) *par*C (E84G, S80I)	IncHI2	none
1057A	4	AMP, GTN, HLFQ	*blaTEM-1b, aadA2, aph(4)-Ia, aph(3')-Ia, aac(3)-Iva, tet(A), tet(M), sul2, sul3, cmlA1, floR,mph(A)*	*gyr*A (S83L, D87N), *par*C (S80I)	IncHI2	ND***
1263A	4	AMP, LLFQ, SXT	*blaTEM-1b, strB, strA, dfrA1, tet(A) sul1, sul2*	*gyr*A (S83L)	IncI2	none

* Acquired resistance to ampicillin (AMP), fluoroquinolones (low level: LLFQ, high level: HLFQ), cotrimoxazole (SXT), gentamicin / tobramycin / netilmicin (GTN) determined by disk diffusion method.
** The detection of the resistances is based on the whole genome sequence data.
*** ND=not done.

Figure S1. Map of the University hospitals of Paris ("Assistance Publique-Hôpitaux de Paris") with Paris *intra-muros* in blue and its suburb outside in white. The six hospitals where the Coli-RED study was performed are indicated. For each hospital, the name of the hospital is provided. For the four suburb hospitals, the code of the district followed by the town are indicated. The number close to each hospital corresponds to the number of beds.

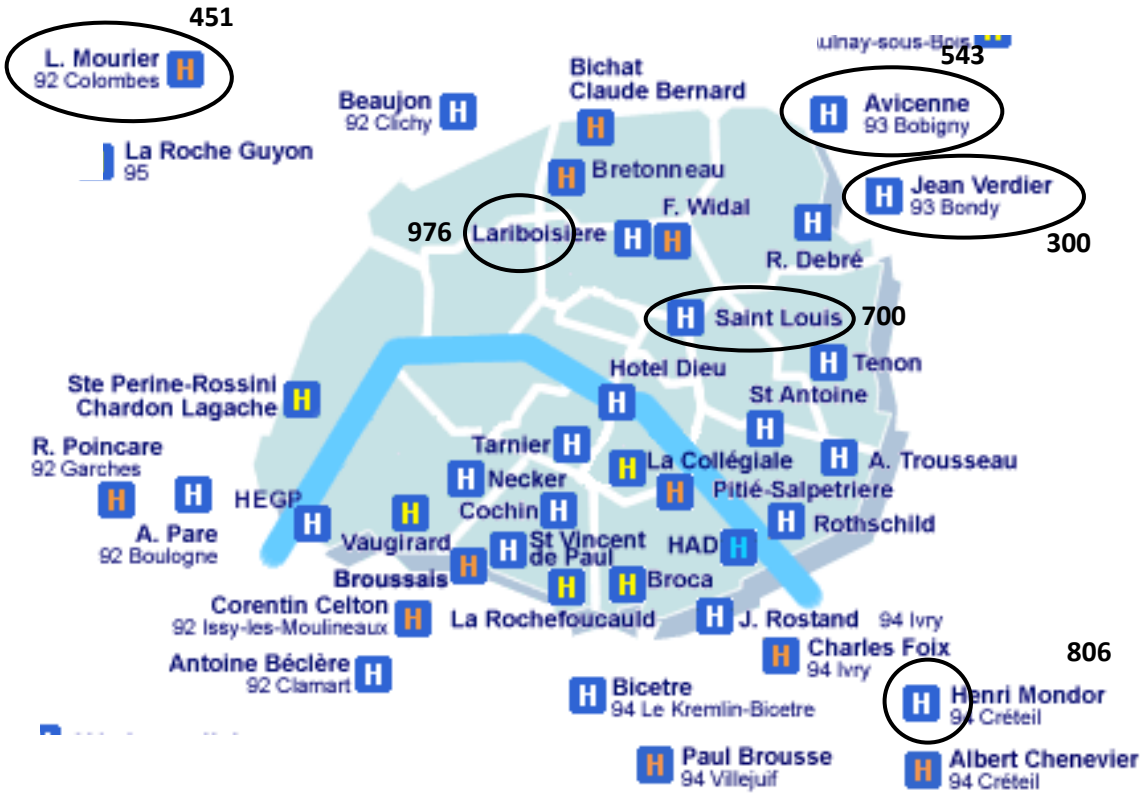
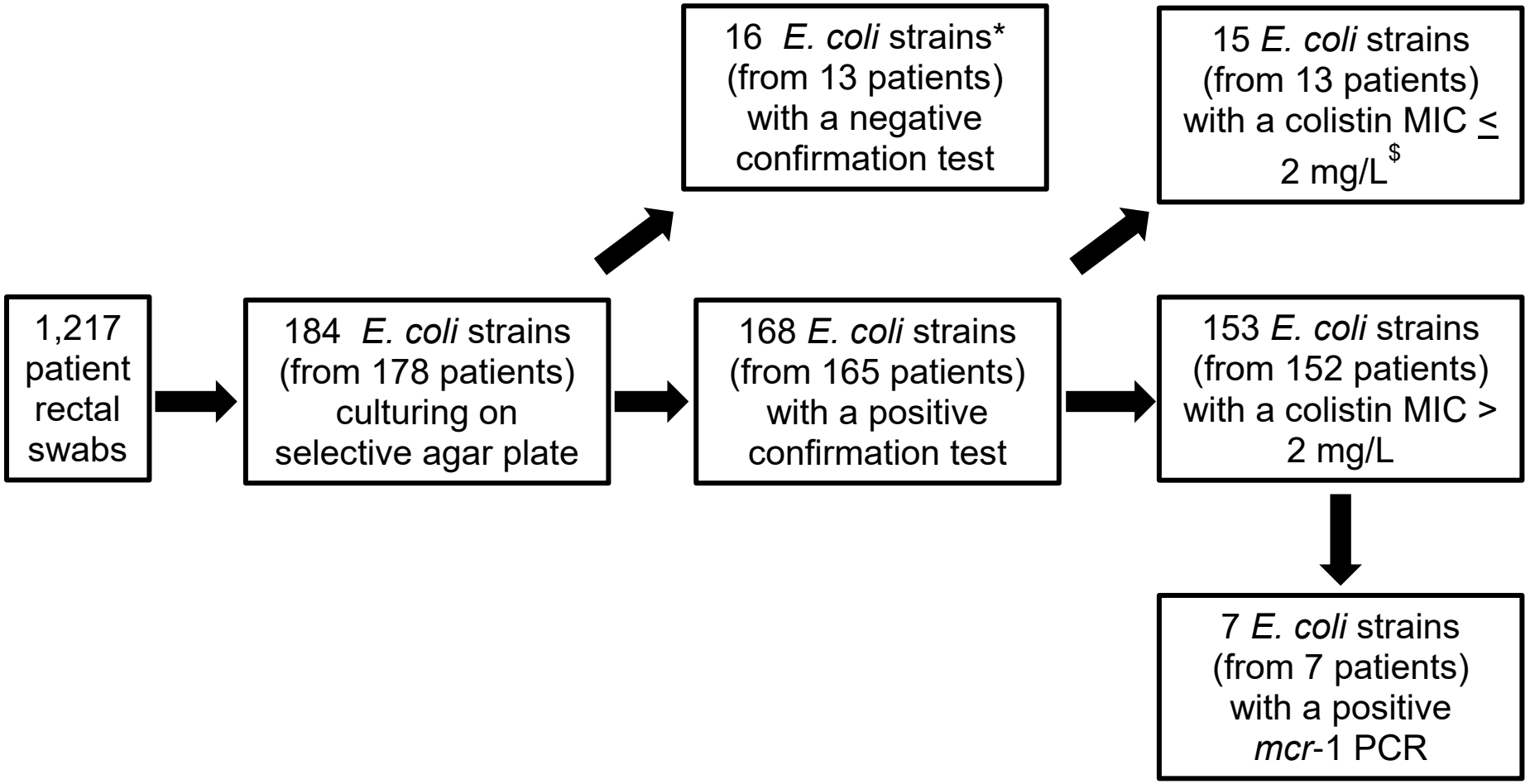

451
L. Mourier
92 Colombes
Beaujon
92 Clichy
Bichat
Claude Bernard
543
Avicenne
93 Bobigny
La Roche Guyon
95
Bretonneau
Jean Verdier
93 Bondy
300
F. Widal
976
Lariboisière
R. Debré
Saint Louis
700
Hotel Dieu
Tenon
Ste Perine-Rossini
Chardon Lagache
St Antoine
R. Poincare
92 Garches
Tarnier
La Collégiale
A. Trousseau
Necker
Pitié-Salpetriere
HEGP
Cochin
Rothschild
A. Pare
92 Boulogne
Vaugirard
St Vincent
de Paul
HAD
Broussais
Broca
Corentin Celton
92 Issy-les-Moulineaux
La Rochefoucauld
J. Rostand
94 Ivry
Charles Foix
94 Ivry
806
Antoine Béclère
92 Clamart
Bicetre
94 Le Kremlin-Bicetre
Henri Mondor
94 Créteil
Paul Brousse
94 Villejuif
Albert Chenevier
94 Créteil

Figure S2. Flow chart of the ColiRED study showing the strategy used for the screening of colistin resistant E. coli strains in 1,217 patients.



* all the 16 strains exhibited a colistin MIC < 2 mg/L and a negative *mcr* PCR

$ all the 15 strains exhibited a negative *mcr* PCR

Figure S3. Genetic environment of the plasmid-borne mcr-1 genes.

Strains are indicated by their ID and ordered as in Table S3. Coding sequences in a window of 5 kbp around mcr-1 gene are represented by arrows. Contig borders are indicated by black vertical lines. The mcr related CDS are in red, pap2 genes in blue (hatched if fragmented), and ISApl1 related sequences in green.

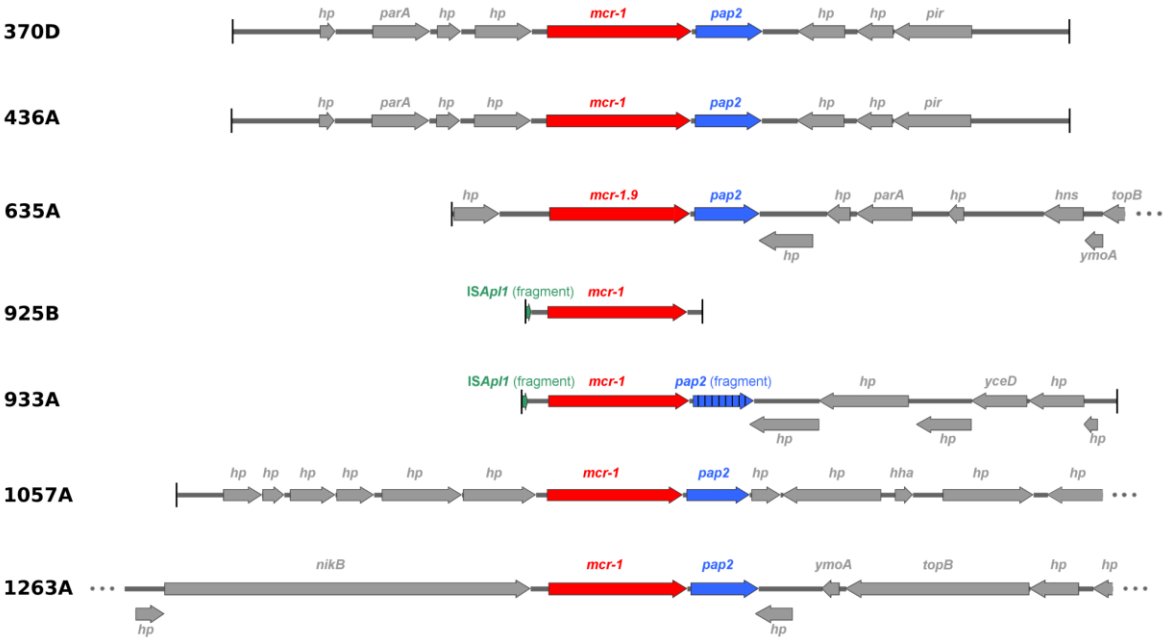

370D
hp
parA
hp
hp
mcr-1
pap2
hp
hp
pir
436A
hp
parA
hp
hp
mcr-1
pap2
hp
hp
pir
635A
hp
mcr-1.9
pap2
hp
parA
hp
hns
topB
hp
ymoA
925B
ISApl1 (fragment)
mcr-1
933A
ISApl1 (fragment)
mcr-1
pap2 (fragment)
hp
yceD
hp
hp
hp
hp
1057A
hp
hp
hp
hp
hp
hp
mcr-1
pap2
hp
hp
hha
hp
hp
1263A
nikB
mcr-1
pap2
ymoA
topB
hp
hp
hp
hp

Table S5. Mutations in pmrA and pmrB genes altering the length of the predicted proteins

Strain ID	ST Warwick	ST Pasteur	Phylogroup	Serotype	Protein	Nucleic variation	Proteic variation	Type of mutation	MIC
130A	131	43	B2	O25b:H4	PmrA	505_510del	V169del/H170del	2 A.A. deletion	8
353A	69	77	D	O17:H18	PmrB	329T<G	L110X	Truncating mutation	8
389A	165	898	A	Unknown:H10	PmrB	329T<G	L110X	Truncating mutation	4
12B	961	882	B2	O4:H5	PmrB	151_412dup	S138KfsX10	Truncating mutation	8
411A	38	8	D	O86:H18	PmrB	143_144delTAinsGGG / 149delAinsCTAT	L48RfsX14	Truncating mutation	8
156A	10	2	A	O8:H4	PmrB	209_238dup	I70_L79dup	10 A.A. duplication	8
619A	38	535	D	O7:H18	PmrB	209_238dup	I70_L79dup	10 A.A. duplication	4
962A	1604	884	B2	O75:H5	PmrB	587_592dup	L199_A200dup	2 A.A. duplication	8
142A	62	757	F	O7:H45	PmrB	200_229del	A67_M76_del	10 A.A. deletion	8
383A	5924	880	B2	O18:H4	PmrB	289_303del	E97_E101del	5 A.A. deletion	16
393A	421	70	B2	O1:H7	PmrB	291_303del	L98_E101del	4 A.A. deletion	8
775A	12	35	B2	O4:H5	PmrB	343_351del	I115_S117del	3 A.A. deletion	16
684A	73	29	B2	O2:H1	PmrB	379_381del	S127del	1 A.A. deletion	32