frontiers

Supplementary Material

Mobile colistin resistance (MCR), extended-spectrum beta-lactamase (ESBL) and multidrug resistance monitoring in *Escherichia coli* (commensal and pathogenic) in pig farming: Need of harmonized guidelines and clinical breakpoints

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PCR reactions

E. coli DNA was extracted from the confluent growth from the fecal swabs plated on lactose MacConkey agar (LMAC, Oxoid) incubated at 37 °C for 18–24 h or from a single overnight-grown colony using the boiling lysis method. Briefly, overnight-grown was picked with a 1 µl inoculation loop and suspended in 600 µl of sterile Milli-Q water. Bacterial suspensions were boiled at 100 °C for 5 min and then centrifuged for 2 min at 11,000 rpm to pellet bacterial debris. The supernatant was used as DNA template in PCR.

All PCR reactions were done in a final reaction volume of 25 µl, using 5 µl of DNA template, 12.5 µl of NZYTaq 2x Green MasterMix, 0.2-0.5 µM of each primer, and up to 25 µl of sterile Milli-Q water. PCR amplifications were performed on Applied Biosystem 2720 Thermal Cycler using an initial heat activation step of 3 min at 94 °C; then 35 cycles of 1 min at 94 °C, 1 min at specific annealing temperature (depending on the primer set used), and 90 min at 72 °C; and an final extension step of 3 min at 72 °C.

PCR products were separated through 1.5% agarose gel (Seakem LE agarose, Lonza) containing Green Safe Premium (Nzytech) (3 µl/100 ml agarose) by convectional electrophoresis and the amplified PCR products were visualized using Gel Doc XR (BioRad, CA).

The primers, the amplicon size and the specific annealing T^a for each PCR reaction are indicated in the Supplementary Tables 1, 2, 3, and 4.

Table S1. Targets and primers associated with diarreagenic pathotypes of *E. coli*

Pathotype	Target	Primers	Nucleotide sequence (5´- 3´)	Size (bp)	Annealing Tª (°C)	Reference
STEC	*stx2e*	Stx2e-F1	CGGAGTATCGGGGAGAGGC	411	58	Scheutz *et al.*, 2012
		Stx2e-R2	CTTCCTGACACCTTCACAGTAAAGGT			
EPEC	*eae*	EAE-V3F	CATTGATCAGGATTTTTCTGGT	510	54	Mora *et al.*, 2011
		EAE-MBR	TCCAGAATAATATTGTTATTACG			
ETEC	*eltA*	LT-A-1	GGCGACAGATTATACCGTGC	696	54-56	Schultsz *et al.*, 1994
		LT-A-2	CCGAATTCTGTTATATATGTC			
ETEC	*estA*	STa-A	ATTTTTATTTCTGTATTGTCTTT	176	50-52	Penteado *et al.*, 2002
		STa-B	GGATTACAACACAGTTCACAGCAGT			
ETEC	*estB*	STb-1	ATCGCATTTCTTCTTGCATC	172	54	Blanco *et al.*, 1997
		STb-2	GGGCGCCAAAGCATGCTCC			
ETEC	F18	F18-F	GTGAAAAGACTAGTGTTTATTC	510	54-56	Imberechts *et al.*, 1992
		F18-R	CTTGTAAGTAACCGCGTAAGC			
ETEC	F4 (K88)	K88-F	GGTGATTTCAATGGTTCGGTC	764	66	Franklin *et al.*, 1996
		K88-R	ATTGCTACGTTCAGCGGAGCG			
ETEC	F5 (K99)	K99-A	CCAGCGCCCGGCAGTAATGACTGC	278	64	Blanco *et al.*, 2006
		K99-B	CCACCATTAGACGGAGCGCGG			
ETEC	F41	F41-A	GGCTATGGAAGACTGGAGAGGG	545	60	Blanco *et al.*, 2006
		F41-RN	GACTGAGGTCATCCCAATTGTGG			García-Meniño *et al.*, 2018
ETEC	F6 (P987)	P987-F	GCGCCCGCTGAAAACAACACCAGC	467	64	Blanco *et al.*, 2006
		P987-R	GTACCGGCCGTAACTCCACCG			

Table S2. Primers used for the detection of *rbf*O25 and *uidA* genes

Target	Primers	Nucleotide sequence (5´- 3´)	Size (bp)	Annealing Tª (°C)	Reference
*rfb*O25b	rfbO25b.r	TGCTATTCATTATGCGCAGC	300	56	Clermont *et al.*, 2008
	rfb.1bis	ATACCGACGACGCCGATCTG			
uidA	uidA-F	GCGTCTGTTGACTGGCAGGTGGTGG	503	60	Gómez-Duarte *et al.,* 2010
	uidA-R	GTTGCCCGCTTCGAAACCAATGCCT			

Table S3. Targets and primers to determine phylogroups

Target	Primers	Nucleotide sequence (5´- 3´)	Size (bp)	Annealing Tª (°C)	Reference
chuA	chuA.1b	ATGGTACCGGACGAACCAAC	288	58	Clermont *et al*., 2013
	chuA.2	TGCCGCCAGTACCAAAGACA			Clermont *et al*., 2000
yjaA	yjaA.1b	CAAACGTGAAGTGTCAGGAG	211		Clermont *et al*., 2013
	yjaA.2b	AATGCGTTCCTCAACCTGTG			
TspE4C2	TspE4C2.1b	CACTATTCGTAAGGTCATCC	152		Clermont *et al*., 2013
	TspE4C2.2b	AGTTTATCGCTGCGGGTCGC			
arpA	AceK.f	AACGCTATTCGCCAGCTTGC	400		Clermont *et al*., 2013
	ArpA1.r	TCTCCCCATACCGTACGCTA			
trpAgpC (C)	trpAgpC.1	AGTTTTATGCCCAGTGCGAG	219	56	Lescat *et al.*, 2013
	trpAgpC.2	TCTGCGCCGGTCACGCCC			
arpA (E)	ArpAgpE.f	GATTCCATCTTGTCAAAATATGCC	301	57	Lescat *et al.*, 2013
	ArpAgpE.r	GAAAAGAAAAAGAATTCCCAAGAG			
trpA	trpBA.f	CGGCGATAAAGACATCTTCAC	489	56	Clermont *et al.*, 2008
	trpBA.r	GCAACGCGGCCTGGCGGAAG			
ybgD (G)	*ybgD.1*	TATGCGGCTGATGAAGGATC	177	59	Clermont *et al.*, 2019
	ybgD.2	GTTGACTAAGCGCAGGTCGA			
cfaB (F)	*cfaB.1*	CTAACGTTGATGCTGCTCTG	384		Clermont *et al.*, 2019
	cfaB.2	TGCTAACTACGCCACGGTAG			

Table S4. Primers used for the detection and / or sequencing of TEM, SHV, CTX-M and MCR genes

Target	Primers	Nucleotide sequence (5´- 3´)	Size (bp)	Annealing Tª (°C)	Reference
bla$_{CTX-M}$	CTX-C3	ATGTGCAGCACCAGTAAAGTGATG	542	55	Mora *et al.*, 2013
	CTX-C4	ACCGCGATATCGTTGGTGGTGCC			
bla$_{CTX-M}$ group1	M13U	GGTTAAAAAATCACTGCGTC	863	60	Saladin *et al.*, 2002
	M13L	TTGGTGACGATTTTAGCCGC			
blaCTX-M-grupo 1	[a]CTX-15-F1	GAAGCTAATAAAAAACACACGTGG	1044-1123	52	Mora *et al.*, 2013
	[a]CTX-15-R	GTATGCGCAAGCGCAGGTGG			
bla$_{CTX-M}$ group9	CTX-M9-F	GTGACAAAGAGAGTGCAACGG	856	64	Simarro *et al.*, 2000
	CTX-M9-R	ATGATTCTCGCCGCTGAAGCC			
bla$_{CTX-M}$ group9	[a]CTX-M9-14-14B-24F	GAATACTGATGTAACACGGA	998	44	García-Meniño *et al.*, 2018
	[a]CTX-M9-R	AGCTGAAGATGTATATCAAG			
bla$_{CTX-M}$ group9	[a]CTX-M9-14-14B-24F	GAATACTGATGTAACACGGA	989	52	García-Meniño *et al.*, 2018
	[a]CTX-M14-24-R	CTGCGTTGTCGGGAAGATACG			
bla$_{CTX-M}$ group9	[a]CTX-M9-14B-F	CCTATACCCGAGGCGCGACAG	1059	44	García-Meniño *et al.*, 2018
	[a]CTX-M9-R	AGCTGAAGATGTATATCAAG			
bla$_{CTX-M}$ group9	[a]CTX-M14-24-F	CTAAATTCTTCGTGAAATAGTG	1049	44	García-Meniño *et al.*, 2018
	[a]CTX-M14-24-R	CTGCGTTGTCGGGAAGATACG			
bla$_{SHV}$	SHV-F2	TTGTCGCTTCTTTACTCGCC	879	64	Mora *et al.*, 2013
	SHV-R2	CCCGGCGATTTGCTGATTTCGC			
mcr-1	mcr1_320bp_fw	AGTCCGTTTGTTCTTGTGGC	320 pb	58	Rebelo *et al.*, 2018
	mcr1_320bp_rev	AGATCCTTGGTCTCGGCTTG			
mcr-2	mcr2_700bp_fw	CAAGTGTGTTGGTCGCAGTT	715 pb		Rebelo *et al.*, 2018
	mcr2_700bp_rev	TCTAGCCCGACAAGCATACC			
mcr-3	mcr3_900bp_fw	AAATAAAAATTGTTCCGCTTATG	929 pb		Rebelo *et al.*, 2018
	mcr3_900bp_rev	AATGGAGATCCCCGTTTTT			
mcr-4	mcr4_1100bp_fw	TCACTTTCATCACTGCGTTG	1116 pb		Rebelo *et al.*, 2018
	mcr4_1100bp_rev	TTGGTCCATGACTACCAATG			
mcr-5	MCR5 FV	ATGCGGTTGTCTGCATTTATC	1644 pb		Borowiak *et al.*, 2017
	MCR5 RV	TCATTGTGGTTGTCCTTTTCTG			

[a]Primers used for sequencing.

Table S5. General parameters of the assembly B2 commensal *E. coli* genome

Isolate	LREC_294
Kmer	131
Roadmap file size	538667191
Total number of contigs	81
N50	319,990
Length of longest contig (bp)	976,860
Total bp in contigs	5,082,346
Number of contigs > 1kb	38
Total bases in contigs > 1kb	5,065,263
Library	334 +/- 103
Coverage	35x
ENA accession number	ERS12564129

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