SUPPLEMENTAL MATERIAL

TEXT S1

Field operation and soil sampling. Fifteen 2-m^2 field-plots, each isolated by fiberglass frames, were selected from two series of plots that were established in the spring of 1999 and 2010, respectively, and described previously (1, 2). The macrolide "MACRO" treatment group consisted of four replicated plots that received annually (2010 – 2014) a spring application of a mixture of erythromycin (ERY), azithromycin (AZI), and clarithromycin (CLN) to achieve concentrations for each drug of ten mg kg^{-1} soil to a depth of 15 cm (1). The control "UTC' group referred to eight plots that never received any antibiotics but that were otherwise managed exactly as described for the treated plots in the same study. The "SCT" treatment group consisted of three plots that have, since 1999, received annually a mixture of antibiotics commonly used in swine production, namely sulfamethazine (SMZ), chlortetracycline (CTC) and tylosin (TYL) (2). During 1999 – 2004, the annual application concentrations for each drug were 1 mg kg^{-1} soil, while the application concentrations were increased to 10 mg kg^{-1} soil for the period of 2005 – 2014. In June of each year treated plots were amended by mixing in mixtures of the antibiotics to 1 kg of soil from each field-plot, followed by incorporation of the amended soil into the corresponding field-plots to a depth of 15 cm using a mechanized rototiller. Plots were cropped continuously to soybeans (*Glycine max* var. Harosoy) during each growing season, and received no further management other than manual weeding. For the purpose of metagenomic fosmid library construction, soil cores were collected from each replicate plot of the chosen treatment using a T-sampler. The soil samples were then pooled and sieved to a maximum particle size of 2 mm, followed by extraction of high-molecular-weight metagenomic DNA either immediately, or

after 4°C storage for less than 24 h. Soil samples were additionally kept at -20°C for future analysis.

Extraction of high-molecular-weight metagenomic DNA from soil. High-molecular-weight metagenomic DNA was extracted from soil sample using an indirect DNA extraction method as described previously (3, 4). Briefly, 100 gram of the sieved soil sample was homogenized in 400 ml of pre-chilled disruption-buffer containing 50 mM Tris-HCl (pH 8.0) and 0.2 M NaCl using a Waring blender in a 4°C room. The homogenization condition consisted of three one-minute period of blending and two one-minute cooling intervals during which the blender-jug was placed in an ice-water bath. The homogenate was centrifuged at 1,000 x g for 15 min at 4°C to recover the bacterial-cell containing supernatant, whereas the sediment was re-homogenized using fresh disruption-buffer and the same condition as described above. After three successive rounds of homogenization, the detached bacterial cell was pelletized from the supernatant by centrifugation (23, 000 x g, 20 min, 4°C), and subsequently washed twice by resuspending in pre-chilled washing-buffer [10 mM Tris-HCl (pH 8.0) and 400 mM of EDTA (pH 8.0)] followed by centrifugation at the same setting. To perform "in-gel" lysis of the recovered bacterial cells, the washed pellet was embedded within 1% SeaPlaque® GTG® low-melting point agarose (Lonza, Rockland, ME) prepared in 1X TBE buffer and cast in strips of 10-well disposable plug moulds (Bio-Rad, Mississauga, ON). After setting, these bacterial cell-containing plugs were extruded from the plug moulds and incubated in a lysis-buffer consisted of 1% (w/v) Sarkosyl, 1% (w/v) sodium deoxycholate, 1 mg/ml lysozyme, 10 mM Tris-HCl (pH 8.0), 0.2 M EDTA (pH 8.0), and 50 mM NaCl, at 37°C for 1 h. The agarose plugs were then transferred to the ESP buffer [1% Sarkosyl, 1 mg/ml proteinase K, and 0.5 M EDTA (pH 8.0)] and incubated further at 55°C for 16 – 18 h. To inactivate proteinase K, the serine protease inhibitor

phenylmethylsulfonyl fluoride was added to the agarose-plugs/ESP buffer mixture from a freshly prepared stock at a final concentration of 1 mM and incubated at room temperature for 1 h. After washing the agarose plugs in a 10 mM TRIS-HCl (pH 8.0) buffer supplemented with 1 mM of EDTA (pH 8.0) for three 15 min periods with mild-agitation, the agarose plugs were stored in a 10 mM Tris-HCl (pH 8.0) buffer supplemented with 50 mM EDTA (pH 8.0) at 4 ^{o}C for an overnight. Pulse-field gel electrophoresis (PFGE), performed with a CHEF-DR® III variable angle system (Bio-Rad) (Run parameters: switch time, 1 – 25 sec; angle, 120^{o}; voltage, 6V cm^{-1}; temperature, 12^{o}C; run time, 16 h), was used to size-fractionate the soil-derived metagenomic DNA entrapped within the treated agarose plugs onto a freshly prepared 1% agarose gel, in 0.5X TBE buffer, with the region encompassing the molecular size of 25 – 70 kb excised thereafter. The size-selected metagenomic DNA was electro-eluted from the excised gel block and concentrated by ethanol precipitation. No additional shearing of DNA was necessary before downstream application, as general handling and purification process was enough to produce fragments with optimal size (25 - 40 kb) for fosmid library construction.

Selection for antibiotic-resistant metagenomic fosmid clones. Diluted metagenomic clone library cultures were plated onto LB-agar plates supplemented with one of the following antibiotics at the indicated concentration: kanamycin (KAN, 50 μg ml^{-1}), gentamicin (GEN, 20 μg ml^{-1}), tetracycline (TET, 4 μg ml^{-1}), chlortetracycline (CTC, 64 μg ml^{-1}), tylosin (TYL, 2,048 μg ml^{-1}), erythromycin (ERY, 256 μg ml^{-1}), azithromycin (AZI, 32 μg ml^{-1}), cefotaxime (CAX, 4 μg ml^{-1}), ceftiofur (CEF, 8 μg ml^{-1}), meropenem (MEM, 0.125 μg ml^{-1}), or ciprofloxacin (CIP, 0.5 μg ml^{-1}). For the selection of sulfonamide-resistant fosmid clones, Iso-Sensitest agar (Oxoid, Nepean, ON) supplemented with sulfamethazine (SMZ, 2,000 μg ml^{-1}) was used. To assess

clonality, fosmid DNA recovered from individual clones selected by using the same antibiotic was digested with *NotI* to distinguish any redundant clones based on their restriction-patterns.

Fosmid sequencing and bioinformatics parameter. To resolve the metagenomic DNA insert sequence of selected fosmids, sequencing libraries were generated using the Nextera XT DNA Library Preparation and Index kits (Illumina, Victoria, BC) with fosmid DNA that were pre-treated with Plasmid-Safe^{TM} ATP-Dependent DNase (Epicentre) to remove any residual chromosomal DNA contaminant. Multiplex sequencings (up to 43 samples in a single run) were performed on a MiSeq desktop sequencer using the MiSeq Reagent Kit v2 500-cycle (Illumina). Prior to assembly, all sequencing reads had pCC1FOS sequence-containing reads removed using Bowtie v. 1.1.1 with the *-v* option (5) on a 64-bit Ubuntu 14.04 installation. *De novo* assembly of the fosmid insert sequences was then performed using the first 100,000 reads and Velvet v. 1.2.10 (6) on the same machine. The chosen k-mer sizes were between 95 and 125 to obtain coverage of ca. 100- to 150- fold. MetaGeneMark (7) was used to identify genes and open-reading frames (ORFs), which were then subjected to functional annotation by HMMER3 (8). ISfinder (9) was utilized in searching for any bacterial insertion sequences and PhyloPthyiaS (10) in classifying the taxonomic origin of metagenomic DNA.

PCR and molecular cloning of ARGs. Generally, PCR amplification was achieved in a 50-µl reaction mixture containing ca. 10 ng of fosmid DNA, 1 U of Phusion high-fidelity DNA polymerase (New England BioLabs Ltd., Pickering, ON), 1X Phusion HF buffer, primers at a final concentration (each) of 0.5 µM, and deoxynucleoside triphosphates (dNTPs) at a final concentration of 200 µM. The mixture was heated for 30 sec at 98^{o}C, followed by 30 cycle of 10 sec at 98^{o}C, 30 sec at 72^{o}C and 15 - 75 sec at 72^{o}C (see ***Table 10***), before finishing with 10 min at 72^{o}C. For restriction endonuclease digestion, ligation, transformation, plasmid isolation, and

agarose gel electrophoresis, standard protocols as described by Sambrook and Russell (11) were used. All plasmid-cloned DNA fragments were sequenced (Robarts Research Institute, London, ON) using universal and custom primers, to verify that no unintended mutations were introduced during PCR.

Proteomics analysis. Overnight *E. coli* culture (2 – 4 ml) with final OD_{600nm} value of ca. 2.0 was pelleted, resuspended in 500 µl of ice-cold ammonium bicarbonate (50 mM), and sonicated using a Q125 sonicator (Qsonica) with the cell suspension kept on ice throughout (six bursts of 15 sec at 40% power with 30 sec resting intervals). Following centrifugation (16,000 x g, 10 min, 4^{o}C) to remove insoluble cell debris and unbroken cell, ~ 100 µg of protein was incubated with 200 µl 0.1% RapiGest^{TM} SF surfactant (Waters Corp.) at 99 ^{o}C for 2 min. After cooling, the sample was reduced by incubating with 5 mM dithiothreitol at 60 ^{o}C for 30 min, and acetylated with 15 mM iodoacetamide in the dark for another 30 min at room temperature. Samples were incubated overnight with MS-grade trypsin protease (1:40) (Thermo-Fisher Scientific), in the presence of 1 mM $CaCl_2$, at 37 ^{o}C with mild agitation. Hydrochloric acid was added (final pH < 2) to precipitate the RapiGest^{TM} detergent, which was subsequently removed by centrifugation. The peptide digests were analyzed using an Easy-nLC 1000 nano system with the Acclaim C18 PepMap™ trap column (100 µm x 2 cm) and analytical column (75 µm x 15 cm), coupled to a Q-Exactive Orbitrap mass spectrometer (Thermo-Fisher Scientific). The flow rate was 300 nL min^{-1} and 10 µL of the protein digest was injected. 97% mobile phase A (LC/MS Optima water, 0.1% formic acid) was decreased to 90% over 4 min. Peptides were eluted with a linear gradient from 10 - 35% mobile phase B (LC/MS Optima acetonitrile 0.1% formic acid) over 70 min followed by 35 - 90% over 4 min and maintained for 16 min. The nanospray voltage was set at 1.95 kV, capillary temperature 275 °C, and S-lens RF level 60. Each sample was analyzed by a

top 10 data-dependent acquisition experiment. The full scan was operated at 70,000 resolution, automatic gain control (AGC) of 1 x e^6 and maximum injection time (IT) of 240 ms. The MS/MS scans were acquired at 17,500 resolution, AGC of 1 x e^5, maximum IT of 110 ms, intensity threshold of 8 x 10^4, normalized collision energy of 27 and isolation window of 2.0 *m/z*. Unassigned, singly and >4 charged peptides were not selected for MS/MS and a 20 sec dynamic exclusion was used. The Thermo *.raw* files were converted to *.mgf* using Proteowizard v2 (12). The MS/MS scans were searched against the target/reverse proteome of *E. coli* MG1655 (Proteome ID UP0000625, accessed April**,** 2016) with an additional 39 annotated gene products derived from fosmid MACRO-AZI 4 using the X!tandem search algorithm (13) operated from the SearchGUI v.2.35 interface (14) and processed in PeptideShaker v1.3.6 (15). In addition, a second search under the same conditions was completed using the *E. coli* MG1655 proteome with protein sequences deduced from all six possible reading frames (including $PPP^{AZI\ 4}$) encompassed by the subcloned fragment of pCL043. A 3 ppm precursor ion mass error and a 0.02 Da product ion error were used along with carbamidomethylation as a constant modification and oxidation of methionine as variable. A 1% FDR rate was used at the protein, peptide and peptide spectrum match level.

TABLE S1 Antibiotic susceptibility of soil metagenomic fosmid isolates derived from *E. coli* EPI300-T1^{R} strain

Fosmid Candidates	Minimal Inhibitory Concentration (μg/mL) [a, b, c]																					
	KAN	GEN	AMI	TET	CTC	OTC	TIG	TYL	ERY	AZI	CLR	LIN	CIP	RIF	PXB	MEM	AMP	FOX	CAZ	CRB	CEF	CAX
pCC1FOS	8	8	4	4	64	4	1	2048	256	16	256	256	0.016	16	0.125	0.03	8	16	2	16	0.5	0.125
Group I																						
UTC-KAN K1	3200		8							-	-	-								-	-	-
SCT-KAN 4	128		16							-	-	-								-	-	-
Group II																						
UTC-SMZ B27			-		-	-				-	-	-								-	-	-
SCT-SMZ 28			-		-	-				-	-	-								-	-	-
SCT-SMZ 29			-		-	-				-	-	-								-	-	-
Group III																						
MACRO-MEM A1			-		-	-				-	-	-				32	256	256	16	32	128	64
MACRO-MEM B2			-		-	-				-	-	-				16	>1024	256	64	>2048	16	8
MACRO-MEM B3			-		-	-				-	-	-				32	>1024	256	256	>2048	32	32
Group IV																						
SCT-TET 7			-	64	1024	32						1024								-	-	-
MACRO-TET B9			-	32	256	32	2					512								-	-	-
MACRO-TET A9			-	32	256	32						1024	0.032							-	-	-
SCT-TET 23			-	16	256	32						4096	0.064							-	-	-
UTC-TET 1			-	8	128	16						512	0.032							-	-	-
Group V																						
MACRO-AZI 1			-		-	-		4096	2048	64		4096	0.25							-	-	-
SCT-ERY 2			-		-	-		4096	512			2048	0.25							-	-	-
MACRO-TYL 17			-		-	-	2	8192				512								-	-	-
MACRO-AZI 4			-		-	-		8192	512	64	512									-	-	-
MACRO-TYL 4			-		-	-		4096	512	64	512									-	-	-
MACRO-TYL 10		4	-		-	-		4096	512	32	512									-	-	-
MACRO-AZI 6			-		-	-			512	64	1024									-	-	-
MACRO-ERY 9			-		-	-			512	64	1024									-	-	-
MACRO-ERY 41			-		-	-			512											-	-	-

Group VI															
MACRO-TET A7	-	64	512	64	16	8192	1024	64		1024			-	-	-
MACRO-TET A6	-	32	256	64	4	4096	512	32		512		8			
MACRO-TET A3	-	32	256	64	4		512			1024			-	-	-
SCT-TET 22	-	32	256	32	4		1024	32		1024			-	-	-
MACRO-TET B1	-	32	256	32	4		1024	32		1024			-	-	-
SCT-TET 24	-	32	256	32	4		1024	32		512			-	-	-
MACRO-TET B2	-	16	256	32	4		512			512			-	-	-
MACRO-ERY 18	-	16	256	32	8	4096	1024	32					-	-	-
MACRO-TET B7	-	32	512	8		4096	512	32	128	1024			-	-	-

[a] Only values that are difference from the pCC1FOS-carrying control strain are included.

[b] AMI, amikacin; AMP, ampicillin; AZI, azithromycin; CAX, cefotaxime; CAZ, ceftazidime; CEF, ceftiofur; CIP, ciprofloxacin; CLR, clarithromycin; CRB, carbenicillin; CTC, chlortetracycline; ERY, erythromycin; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; LIN, lincomycin; MEM, meropenem; OTC, oxytetracycline; PXB, polymyxin; RIF, rifampicin; TET, tetracycline; TIG, tigecycline; TYL, tylosin.

[c] - , MIC values not determined.

TABLE S2 Elements inferring antibiotic resistance and gene mobility identified bioinformatically from soil-derived metagenomic fosmid clones

Fosmid	ORF#	Description of best blastX match [bacterial species]	Predicted mechanism [a]	Sequence identity (%)	Sequence coverage (%)	Accession no.
Group I						
SCT-KAN 4	23	daunorubicin resistance protein DrrA family ABC transporter ATP-binding protein [*Planomonospora sphaerica*]	Efflux	69%	96%	GAT66031.1
	24	multidrug ABC transporter permease [*Planomonospora sphaerica*]	Efflux	70%	93%	GAT66030.1
Group II						
SCT-SMZ 28	5	MULTISPECIES: integrase [Proteobacteria]	LGT	100%	99%	WP_000954590.1
	6	regulatory protein repA [*Shigella dysenteriae* 1617]	LGT	100%	95%	EFP73965.1
	7	MULTISPECIES: replication protein C [Proteobacteria]	LGT	100%	99%	WP_000743064.1
	9	conjugal transfer protein TrbJ [uncultured bacterium IN-11]	LGT	100%	70%	AMP42399.1
	10	Arsenate reductase [*Gallibacterium anatis*]	Enzymatic	99%	99%	WP_039159282.1
	11	Arsenic resistance protein ArsH [*Shigella dysenteriae* WRSd3]	Enzymatic	100%	99%	ESU81725.1
	13	MULTISPECIES: resolvase [Proteobacteria]	LGT	100%	99%	WP_001366550.1
	15	MULTISPECIES: transposase [Proteobacteria]	LGT	100%	99%	WP_001120888.1
SCT-SMZ 29	B1	superfamily II DNA/RNA helicase [*Nitrosomonas eutropha]*	LGT	65%	100%	WP_011634393.1
	B2	restriction endonuclease [*Nitrosomonas eutropha*]	LGT	59%	99%	WP_011634392.1
	B20	replicative DNA helicase [*Halobacteriovorax marinus*]	LGT	42%	95%	WP_014245262.1
	B21	integrase [*Gemmatimonas sp.* SG8_17]	LGT	42%	91%	KPJ94178.1
Group III						
MACRO-MEM A1	24	hydrolase beta-lactamase-like protein [*Janthinobacterium sp.* HH01]	Enzymatic	58%	93%	ELX11131.1
	32	metallo-beta-lactamase domain protein [*Candidatus Thiomargarita nelsonii*]	Enzymatic	46%	97%	OAD22843.1

MACRO-MEM B2	16	MexE family multidrug efflux RND transporter periplasmic adaptor subunit [*Pseudoduganella violaceinigra*]	Efflux	92%	96%	WP_035374780.1
	17	multidrug efflux RND transporter permease subunit [*Pseudoduganella violaceinigra*]	Efflux	97%	99%	WP_028103618.1
	18	RND transporter [*Pseudoduganella violaceinigra*]	Efflux	94%	99%	WP_028103617.1
	26	MULTISPECIES: Ku protein [*Duganella*]	LGT	96%	78%	WP_055926129.1
MARCO-MEM B3	10	MULTISPECIES: Ku protein [*Duganella*]	LGT	96%	78%	WP_055926129.1
	25	MBL fold metallo-hydrolase [*Duganella sp.* Root1480D1]	Enzymatic	95%	99%	WP_057264869.1
Group IV						
SCT-TET 7	10	DNA recombination/repair protein RecA [*Neorhizobium galegae*]	LGT	98%	95%	WP_046636404.1
SCT-TET 23	11	MBL fold metallo-hydrolase [*Leifsonia sp.* Root227]	Enzymatic	49%	98%	WP_055892941.1
UTC-TET 1	25	glyoxalase/bleomycin resistance protein/dioxygenase [*Arthrobacter crystallopoietes*]	Enzymatic	68%	94%	WP_005268767.1
Group V						
MACRO TYL 17	19	aminoglycoside phosphotransferase [*Thermus oshimai*]	Enzymatic	52%	95%	WP_015065425.1
MACRO AZI 4	12	multidrug ABC transporter ATPase/permease [*Gordonibacter pamelaeae*]	Efflux	43%	94%	WP_015540284.1
MACRO-TYL 10	6	transposase [*Desulfotomaculum kuznetsovii*]	LGT	35%	97%	WP_013824420.1
	15	MBL fold metallo-hydrolase [*Parachlamydia acanthamoebae*]	Enzymatic	52%	95%	WP_013924979.1
MACRO-ERY 41	15	Ku protein [*Chlamydia sp. 'Diamant'*]	LGT	54%	84%	WP_032124496.1
	16	DNA ligase D [*Cytophaga aurantiaca*]	LGT	47%	99%	WP_018344657.1
	22	EmrB/QacA subfamily drug resistance transporter [Candidatus Saccharibacteria bacterium_44_17]	Efflux	57%	94%	AKM80266.1
Group VI						
MACRO TET A6	6	DNA ligase D [*Cytophaga aurantiaca*]	LGT	48%	99%	WP_018344657.1
	7	Ku protein [*Protochlamydia naegleriophila*]	LGT	53%	99%	WP_059062007.1
MACRO-TET A7	42	putative exporter of polyketide antibiotics- like protein [*Thermomonospora curvata*]	Efflux	46%	95%	WP_012854905.1
MACRO TET A3	10	repair protein RadC protein	LGT	48%	78%	AKM80549.1

		[*Candidatus Saccharibacteria* bacterium GW2011_GWC2_44_17]				
MACRO ERY 18	35	recombination protein RecR [*Candidatus Saccharibacteria* bacterium GW2011_GWA2_46_10]	LGT	72%	97%	KKU19805.1
	38	Replicative DNA helicase [*Candidatus Saccharibacteria* bacterium GW2011_GWA2_46_10]	LGT	68%	96%	KKU19802.1
MACRO TET B7	3	recF protein [*Acidobacteria* bacterium OLB17]	LGT	67%	97%	KXK01516.1
	4	DNA gyrase subunit B [*Acidobacteria* bacterium OLB17]	LGT	81%	99%	KXK01515.1
	20	beta-lactamase [*Acidobacteria* bacterium OLB17]	Enzymatic	57%	81%	KXK01503.1

[a] LGT, lateral gene transfer

TABLE S3 Taxonomic assignment of the fosmid-cloned metagenomic DNA using PhyloPythiaS

Fosmid candidate	superkingdom	phylum	class	order	family	genus
Group I						
UTC-KAN K1						
a:	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	**Escherichia**
b:	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	**Escherichia**
SCT-KAN 4	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	**Hyphomonas**
Group II						
UTC-SMZ B27	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	**Candidatus Koribacter**
SCT-SMZ 28	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	**Sphingopyxis**
SCT-SMZ 29						
a:	Bacteria	Firmicutes	Clostridia	**Clostridiales**		
b:	Bacteria	Lentisphaerae	Lentisphaeria	Lentisphaerales	Lentisphaeraceae	**Lentisphaera**
Group III						
MACRO-MEM A1	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	**Collimonas**
MACRO-MEM B2	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	**Collimonas**
MACRO-MEM B3	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	**Janthinobacterium**
Group IV						
SCT-TET 7	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	**Ochrobactrum**
MACRO-TET B9	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	**Providencia**
MACRO-TET A9	Bacteria	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	**Nitrosomonas**
SCT-TET 23	Bacteria	Actinobacteria	Actinobacteria	Coriobacteriales	**Coriobacteriaceae**	
UTC-TET 1	**Bacteria**					
Group V						
MACRO-ERY 4						
a:	Bacteria	Actinobacteria	Actinobacteria	Coriobacteriales	Coriobacteriaceae	**Collinsella**
b:	Bacteria	Actinobacteria	**Actinobacteria**			
SCT-ERY 2	Bacteria	Acidobacteria	Blastocatellia	-	-	**Candidatus Chloracidobacterium**
MACRO-TYL 17	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	**Citromicrobium**
MACRO-AZI 4	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	**Taylorella**
MACRO-TYL 4	Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	**Thermobacillus**

MACRO-TYL 10	Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	Simkaniaceae	**Simkania**
MACRO-AZI 6	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	**Acidovorax**
MACRO-ERY 9	Bacteria	Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	-	**Moorea**
MACRO-ERY 41	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Cellvibrionaceae	**Teredinibacter**
Group VI						
SCT-TET 24	Bacteria	Actinobacteria	Actinobacteria	Coriobacteriales	**Coriobacteriaceae**	
MACRO-TET A6	Bacteria	Actinobacteria	Actinobacteria	Coriobacteriales	Coriobacteriaceae	**Atopobium**
MACRO-TET A7	Bacteria	Actinobacteria	Actinobacteria	Coriobacteriales	Coriobacteriaceae	**Atopobium**
MACRO-TET B1	Bacteria	Actinobacteria	Actinobacteria	Coriobacteriales	**Coriobacteriaceae**	
MACRO-ERY 18	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	**Veillonella**
MACRO-TET A3	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	**Thermosinus**
MACRO-TET B7	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	**Hyphomicrobium**
SCT-TET 22	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Cellvibrionaceae	**Teredinibacter**
MACRO-TET B2	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	**Psychrobacter**

TABLE S4 General macrolide resistance conferred by *ppp*$^{AZI\ 4}$ is EF-P independent in *E. coli*

Strain	Vector	Genotype [a]		Minimal Inhibitory Concentration (μg/ml) [b]					
		ppp$^{AZI\ 4}$	*efp*	TYL	ERY	AZI	CLN	TLT	JOS
BW25113									
	pCF430	-	+	4096	512	64	512	64	1024
	pCL047	+	+	**16384**	**1024**	**256**	**1024**	**256**	**2048**
MW1014									
	pCF430	-	-	4096	512	32	256	64	1024
	pCL047	+	-	**16384**	**1024**	**128**	**512**	**256**	**2048**

[a] -, absent; +, present.

[b] MIC values higher than the corresponding empty vector control's were bolded. AZI, azithromycin; CLN, clarithromycin; ERY, erythromycin; JOS, josamycin; TLT, telithromycin; TYL, tylosin.

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