

In vitro assessment of antimicrobial activity of phytobiotics composition containing menthol, limonen, thymol, p-cymene, trans-anethole, methyl salicylate, terpinen-4-ol and γ -terpinene towards of Avian Pathogenic Escherichia coli (APEC) and other E. coli strains isolated from broiler chickens.

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Table S1. Antibiotic phenotype pattern, distribution of resistance genes, virulence genes and Multiple Antibiotic Resistance Index (MAR Index) amongst *Escherichia coli* isolates from poultry samples.

Sample origin	Antibiotic phenotype pattern	Resistance Genes	Virulence Genes	MAR index
chick	AMX	<i>blasHV</i>	<i>astA, iss, irp2, cvi/cva, iucD, vat, iutA</i>	0.04
	AMX-CPH-DOX-OXY-FLR	<i>blasHV, tetA, tetB, floR</i>	<i>astA, iss, irp2, papC, iucD, vat, ompT</i>	0.2
	AMX-STR-NOR-DOX-OXY	<i>blasHV, aadA, strA/strB, tetA, tetB</i>	<i>astA, iss, irp2, papC, tsh, vat, ompT</i>	0.2
	AMX-CPH-STR-NOR-TR/SMX	<i>blasHV, aadA, strA/strB</i>	<i>astA iss, irp2, papC, iucD, vat, iutA</i>	0.2
	AMX-STR-NOR-FLR-TR/SMX	<i>blasHV, aadA, strA/strB, floR</i>	<i>astA iss, irp2, cvi/cva, iucD, tsh, iutA</i>	0.24
	AMX-STR-DOX-OXY-LIN/SP-TR/SMX	<i>blasHV, aadA, strA/strB, tetA, tetB, sul1</i>	<i>astA iss, irp2, papC, cvi/cva, iucD, vat</i>	0.28
	AMX-CPH-GEN-NOR-DOX-OXY-TR/SMX	<i>blasHV, aadB, tetA, tetB, sul1</i>	<i>astA iss, irp2, cvi/cva, iucD, tsh, iutA</i>	0.28
	AMX-CPH-STR-ENR-NOR-LIN/SP-TR/SMX	<i>blasHV, aadA, strA/strB, sul1, sul2</i>	<i>astA, iss, irp2, cvi/cva, iucD, tsh, iutA</i>	0.28
	AMX-AMX/CL-STR-NOR-DOX-OXY-TR/SMX	<i>blasHV, aadA, strA/strB, tetA, tetB, sul1</i>	<i>astA, iss, irp2, papC, iucD, vat, iutA</i>	0.28
	AMX-GEN-STR-NOR-DOX-OXY-LIN/SP-TR/SMX	<i>blasHV, aadB, aadA, strA/strB, tetA, tetB, sul1, sul2</i>	<i>astA, iss, irp2, cvi/cva, iucD, tsh, iutA</i>	0.32
APEC	AMX	<i>blasHV</i>	<i>astA, iss, irp2, cvi/cva, iucD, vat, iutA</i>	0.04
	ENR-NOR	-	<i>astA, iss, irp2, cvi/cva, iucD, tsh, iutA</i>	0.08
	DOX-OXY	<i>blasHV, tetA, tetB</i>	<i>astA, iss, irp2, papC, cvi/cva, iucD, iutA</i>	0.08
	AMX-CPH-STR	<i>blasHV, aadA, strA/strB</i>	<i>astA, iss, irp2, papC, cvi/cva, iucD, iutA</i>	0.12
	AMX-CPH-ENR-NOR	<i>blasHV</i>	<i>astA, iss, irp2, cvi/cva, iucD, tsh, iutA</i>	0.16
	AMX-CPH-STR-NOR-TR/SMX	<i>blasHV, aadA, strA/strB, sul1, sul2</i>	<i>astA, iss, irp2, papC, iucD, vat, iutA</i>	0.2
	AMX-CPH-DOX-OXY-FLR	<i>blasHV, tetA, tetB, floR</i>	<i>astA iss, irp2, papC, iucD, vat, ompT</i>	0.2
	AMX-CPH-STR-LIN/SP-TR/SMX	<i>blasHV, aadA, strA/strB, sul1, sul2, dfrA1</i>	<i>astA, iss, irp2, cvi/cva, iucD, iutA, ompT</i>	0.2
	AMX-STR-NOR-FLR-TR/SMX	<i>blasHV, aadA, strA/strB, floR, sul1, dfrA1</i>	<i>astA, iss, irp2, cvi/cva, iucD, tsh, iutA</i>	0.2
	AMX-STR-NOR-DOX-OXY	<i>blasHV, aadA, strA/strB</i>	<i>astA, iss, irp2, papC, tsh, vat, ompT</i>	0.2
chicken	NOR-DOX-OXY-FLR-LIN/SP	<i>tetA, tetB, floR</i>	<i>astA, iss, irp2, papC, cvi/cva, iucD, iutA</i>	0.2
	AMX-STR-DOX-OXY-LIN/SP-TR/SMX	<i>blasHV, aadA, strA/strB, tetA, tetB, sul1, sul2, sul3</i>	<i>astA, iss, irp2, papC, cvi/cva, iucD, vat</i>	0.24

AMX-AMX/CL-STR-NOR-DOX-OXY-TR/SMX	<i>blasHV, aadA, strA/strB, tetA, tetB, sul1, sul2, dfrA10</i>	<i>astA, iss, irp2, papC, iucD, vat, iutA</i>	0.28
AMX-CPH-GEN-NOR-DOX-OXY-TR/SMX	<i>blasHV, aadB, tetA, tetB, sul1, sul2, sul3</i>	<i>astA, iss, irp2, cvi/cva, iucD, tsh, iutA</i>	0.28
AMX-CPH-STR-ENR-NOR-LIN/SP-TR/SMX	<i>blasHV, aadA, strA/strB, sul1, sul2, sul3, dfrA1</i>	<i>astA, iss, irp2, cvi/cva, iucD, tsh, iutA</i>	0.28
AMX-CPH-STR-DOX-OXY-FLR-TR/SMX	<i>blasHV, aadA, strA/strB, tetA, tetB, floR, sul1, sul2, sul3</i>	<i>astA, iss, irp2, papC, iucD, tsh, iutA</i>	0.28
AMX-CPH-STR-DOX-OXY-FLR-TR/SMX	<i>blasHV, aadA, strA/strB, tetA, tetB, floR, sul1, sul2</i>	<i>astA, iss, irp2, cvi/cva, iucD, tsh, vat</i>	0.28
AMX-STR-DOX-OXY-FLR-LIN/SP-TR/SMX	<i>blasHV, aadA, strA/strB, tetA, tetB, floR, sul1, sul2, sul3, dfrA12</i>	<i>astA, iss, irp2, cvi/cva, iucD, tsh, iutA</i>	0.28
AMX-CPH-STR-DOX-OXY-FLR-LIN/SP-TR/SMX	<i>blasHV, aadA, strA/strB, tetA, tetB, floR, sul2, sul3</i>	<i>astA, iss, irp2, papC, iucD, vat, iutA</i>	0.32
AMX-GEN-STR-NOR-DOX-OXY-LIN/SP-TR/SMX	<i>blasHV, aadB, aadA, strA/strB, tetA, tetB, sul1, sul2, sul3</i>	<i>astA, iss, irp2, cvi/cva, iucD, tsh, iutA</i>	0.32
AMX-STR-ENR-NOR-DOX-OXY-FLR-LIN/SP	<i>blasHV, aadA, strA/strB, tetA, tetB, floR</i>	<i>astA, iss, irp2, papC, iucD, tsh, vat, iuaT</i>	0.32
AMX-CPH-STR-ENR-NOR-DOX-OXY-FLR-TR/SMX	<i>blasHV, aadA, strA/strB, tetA, tetB, floR sul1, sul2, sul3</i>	<i>astA, iss, irp2, papC, iucD, vat, iutA</i>	0.36
AMX-CPH-STR-ENR-NOR-DOX-OXY-FLR-TR/SMX	<i>blasHV, aadA, strA/strB, tetA, tetB, floR sul1, sul2, sul3</i>	<i>astA, iss, irp2, papC, iucD, tsh, vat,</i>	0.36
AMX-CPH-ENR-NOR-DOX-OXY-FLR-LIN/SP-TR/SMX	<i>blasHV, tetA, tetB, floR dfrA1, dfrA10</i>	<i>astA, iss, irp2, cvi/cva, iucD, tsh, iutA</i>	0.36

Letter abbreviations correspond to the individual antibiotics according to list: amoxicillin (AMX), amoxicillin and clavulanic acid (AMX/CL), cefazirin (CPH), gentamicin (GEN), nstreptomycin (STR), enrofloxacin (ENR), norfloxacin (NOR), docycycline (DOX), oxytetracycline (OXY), florfenicol (FLR), lincomycin/specinicin (LIN/SP), trimethoprim-sulfamethoxazole (TR/SMX).

Table S2 Description of primer sets, annealing temperature and product size for antimicrobial genes

Multiplex PCR or single PCR	Gene/antibiotic	Primer sequences 5' - 3'	Annealing temperature	PCR Product Size (bp)
Multiplex 1	<i>aadA</i>	F - GTG GAT GGC GGC CTG AAG CC	63 °C	525 bp
	streptomycin	R -AAT GCC CAG TCG GCA GCG		
Multiplex 1	<i>strA/strB</i>	F - ATG GTG GAC CCT AAA ACT CT	63 °C	893 bp
	streptomycin	R - CGT CTA GGA TCG AGA CAA AG		
Multiplex 2	<i>aphA1</i>	F - ATG GGC TCG CGA TAA TGT C	55 °C	634 bp
	neomycin	R - CTC ACC GAG GCA GTT CCA T		
Multiplex 2	<i>aphA2</i>	F - GAT TGA ACA AGA TGG ATT GC	55 °C	347 bp
	neomycin	R - CCA TGA TGG ATA CTT TCT CG		
Multiplex 2	<i>aadB</i>	F - GAG GAG TTG GAC TATGGA TT	55 °C	208 bp
	gentamicin	R - CTT CAT CGG CAT AGT AAA AG		
Multiplex 3	<i>tetA</i>	F - GGC GGT CTT CTT CAT CAT GC	63 °C	502 bp
	tetracycline	R -CGG CAG GCA GAG CAA GTA GA		
Multiplex 3	<i>tetB</i>	F - CGC CCA GTG CTG TTG TTG TC	63 °C	173 bp
	tetracycline	R -CGC GTT GAG AAG CTG AGG TG		
Multiplex 4	<i>sul1</i>	F - CGG CGT GGG CTA CCT GAA CG	66 °C	433 bp
	sulfamethoxazole	R - GCC GAT CGC GTG AAG TTC CG		
Multiplex 4	<i>sul2</i>	F - CGG CAT CGT CAA CAT AAC CT	66 °C	721 bp
	sulfamethoxazole	R -TGT GCG GAT GAA GTC AGC TC		
Single PCR	<i>sul3</i>	F - GGGAGCCGCTTCCAGTAAT	60 °C	500 bp
	sulfamethoxazole	R- TCCGTGACACTGCAATCATTA		
Single PCR	<i>dfrA1</i>	F - CAATGGCTGTTGGTGGAC	62 °C	253 bp
	<i>trimethoprim</i>	R - CCGGCTCGATGTCTATTGT		
Single PCR	<i>dfrA10</i>	F - TCAAGGCAAATTACCTTGGC	59°C	433 bp
	<i>trimethoprim</i>	R - ATCTATTGGATCACCTACCC		
Single PCR	<i>dfrA12</i>	F - TTGCGAGACTCACTGAGGG	63 °C	330 bp
	trimethoprim	R - CGGTTGAGACAAGCTCGAAC		
Single PCR	<i>floR</i>	F - CACGTTGAGCCTCTATATGG	61 °C	888 bp
	<i>florfenicol</i>	R - ATGCAGAAGTAGAACGCGAC		
Multiplex 5	<i>blatem</i>	F - TTAACTGGCGAACTACTTAC	55 °C	247 bp
	ampicillin	R - GTCTATTCTGTTCATCCATA		
Multiplex 5	<i>blasthv</i>	F - AGGATTGACTGCCTTTTG	55 °C	393 bp
	amoxicillin	R - ATTGCTGCTATTGCTCG		
Multiplex 5	<i>blacMY-2</i>	F - GACAGCCTCTTCTCCACA	55 °C	1000 bp
	ceftiofur	R - TGGACACGAAGGCTACGTA		
Single PCR	<i>blapse-1</i>	F- GCAAGTAGGGCAGGCAATCA	60 °C	461 bp
	ampicillin	R- GAGCTAGATAGATGCTCACAA		
Single PCR	<i>blactx-m</i>	F- CGCTTGCGATGTGCAG	60 °C	585 bp
		R- ACCGCGATATCGTTGGT		