



**Supplementary Materials:**

**Table 1.** Primer sequences for PCR screening and Sanger sequencing.

Primer name	Resistance genes	Sequence 5'---3'	Product length	Reference
TEM u FWD TEM u REV	<i>bla</i> <sub>TEM</sub>	ATGAGTATTCAACATTTCCG TTAATCAGTGAGGCACCTAT	851 bp	1
SHV u FWD SHV u REV	<i>bla</i> <sub>SHV</sub>	GCAAAACGCCGGTTATTC GGTTAGCGTTGCCAGTGCT	940 bp	1
CTX-M-9 FWD CTX-M-9 REV	<i>bla</i> <sub>CTX-M-9-group</sub>	GCAGTACAGCGACAATACCG TATCATTGGTGGTGCCGTAG	356 bp	1
CTX-M-u FWD CTX-M-u REV	<i>bla</i> <sub>CTX-M-1&amp;2-group</sub>	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	551 bp	1
CTX-M-ges FWD CTX-M-ges REV	<i>bla</i> <sub>CTX-M-1-group</sub>	GTTTCGTCTCTCCAGAATAAGG CAGCACTTTGCCGTCTAAG	968 bp	2
CTX-M-21 FWD CTX-M-21 REV	<i>bla</i> <sub>CTX-M-9-group</sub>	ACACGGATTGACCGTATTGG ATGATTCTCGCCGCTGAAG	889 bp	This study
CTX-M-8 FWD CTX-M-8 REV	<i>bla</i> <sub>CTX-M-8-group</sub>	TAACGCACAGACGCTCTACC TGGCTGGGTGAAGTAAGTGAC	637 bp	4
CTX-M-25 FWD CTX-M-25 REV	<i>bla</i> <sub>CTX-M-25-group</sub>	GCGATGTTAATGACGACAGC AACCGTCGGTGACAATTCTG	847 bp	4
NDM FWD NDM REV	<i>bla</i> <sub>NDM</sub>	CTGAGCACCCGATTAGCC GGGCCGTATGAGTGATTGC	754 bp	3
VIM u FWD VIM u REV	<i>bla</i> <sub>VIM</sub>	AGTGGTGAGTATCCGACAG ATGAAAGTGCCTGGAGAC	261 bp	1
KPC u FWD KPC u REV	<i>bla</i> <sub>KPC</sub>	CAGCTCATTCAAGGGCTTTC AGTCATTTGCCGTGCCATAC	533 bp	1
OXA-48 FWD OXA-48 REV	<i>bla</i> <sub>OXA-48</sub>	AAATCACAGGGCGTAGTTGTG GACCCACCAGCCAATCTTAG	555 bp	1
CMY FWD CMY REV	<i>bla</i> <sub>CMY</sub>	TCTGCTGCTGACAGCCTCT CTCGACACGGACAGGGTTAG	1147 bp	4
QnrA FWD QnrA REV	<i>qnrA</i>	ATTTCTCAGCCAGGATTTG GGCAAAGGTTAGGTCACAG	513 bp	2
QnrB FWD QnrB REV	<i>qnrB</i>	ATGACGCCATTACTGTATAA GATCGCAATGTGTGAAGTTT	562 bp	2

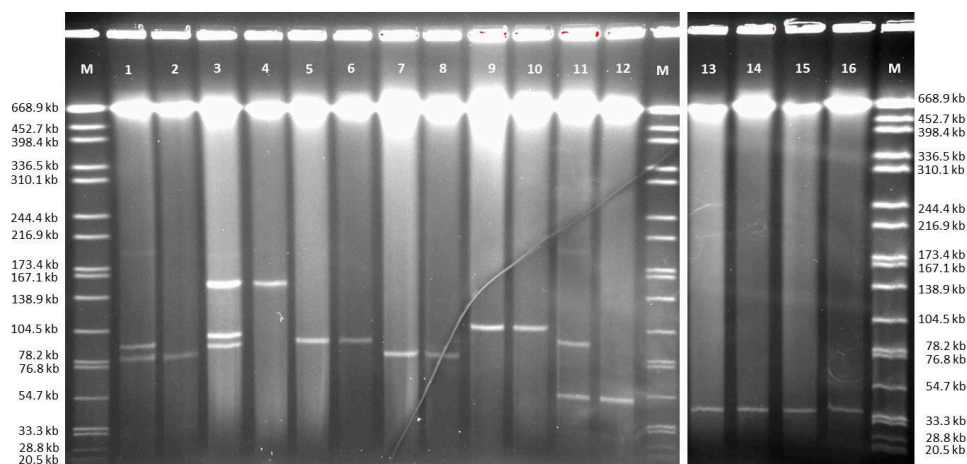
QnrS FWD	<i>qnrS</i>	GGCACCACAACCTTTTCAC	619 bp	2
QnrS REV		CAACAATACCCAGTGCTTCG		
AAC(6')-Ib-cr FWD	<i>aac-like</i>	TTGCGATGCTCTATGAGTGGCTA	482 bp	5
AAC(6')-Ib-cr REV		CTCGAATGCCTGGCGTGTTT		
LAP FWD	<i>bla<sub>LAP</sub></i>	CAATACAAAGCACAGAAGACC	858 bp	6
LAP REV		CCGATCCCTGCAATATGCTC		
Mcr-1 FWD	<i>mcr-1</i>	CGGTCAGTCCGTTTGTTTC	309 bp	7
Mcr-1 REV		CTTGGTCGGTCTGTAGGG		
Mcr-2 FWD	<i>mcr-2</i>	TGTTGCTTGCGGATTGGA	568 bp	8
Mcr-2 REV		AGATGGTATTGTTGGTTGCTG		
Mcr-3 FWD	<i>mcr-3</i>	TTGGCACTGTATTTGCATTT	542 bp	9
Mcr-3 REV		TTAACGAAATTGGCTGGAACA		
Mcr-4 FWD	<i>mcr-4</i>	ATTGGGATAGTCGCCTTTTT	487 bp	10
Mcr-4 REV		TTACAGCCAGAATCATTATCA		
Mcr-5 FWD	<i>mcr-5</i>	TATCTCGACAAGGCCATGCTG	613 bp	11
Mcr-5 REV		GAATCTGGCGTTTCGTCGTAGT		
Mcr-6 FWD	<i>mcr-6</i>	GTCCGGTCAATCCCTATCTGT	556 bp	12
Mcr-6 REV		ATCACGGGATTGACATAGCTAC		
Mcr-7 FWD	<i>mcr-7</i>	TGCTCAAGCCCTCTTTTCGT	892 bp	12
Mcr-7 REV		TTCATCTGCGCCACCTCGT		
Mcr-8 FWD	<i>mcr-8</i>	AACCGCCAGAGCACAGAATT	667 bp	13
Mcr-8 REV		TTCCCCCAGCGATTCTCCAT		

### References Table S1

- Gröbner, S.; Linke, D.; Schutz, W.; Fladerer, C.; Madlung, J.; Autenrieth, I. B.; Witte, W.; Pfeifer, Y., Emergence of carbapenem-non-susceptible extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolates at the university hospital of Tübingen, Germany. *J Med Microbiol* 2009, 58, (Pt 7), 912-22.
- Pfeifer, Y.; Matten, J.; Rabsch, W., *Salmonella enterica* serovar Typhi with CTX-M beta-lactamase, Germany. *Emerg Infect Dis.* 2009, 15, (9), 1533-5.
- Pfeifer, Y.; Wilharm, G.; Zander, E.; Wichelhaus, T. A.; Gottig, S.; Hunfeld, K. P.; Seifert, H.; Witte, W.; Higgins, P. G., Molecular characterization of bla<sub>NDM-1</sub> in an *Acinetobacter baumannii* strain isolated in Germany in 2007. *J Antimicrob Chemother* 2011, 66, (9), 1998-2001.
- Eller, C.; Simon, S.; Miller, T.; Frick, J. S.; Prager, R.; Rabsch, W.; Guerra, B.; Werner, G.; Pfeifer, Y., Presence of beta-lactamases in extended-spectrum-cephalosporin-resistant *Salmonella enterica* of 30 different serovars in Germany 2005-11. *J Antimicrob Chemother* 2013, 68, (9), 1978-81.
- Park C.H.; Robicsek, A.; Jacoby, G.A.; Daniel Sahn, D.; Hooper, D.C., Prevalence in the United States of aac(6<sub>-</sub>)-Ib-cr Encoding a Ciprofloxacin-Modifying Enzyme. *Antimicrob Agents Chemother*, 2006, 50, 3953-3955.
- Huang, Z.; Mi, Z.; Wang, C., A novel beta-lactamase gene, LAP-2, produced by an *Enterobacter cloacae* clinical isolate in China. *J Hosp Infect* 2008, 70, (1), 95-6.

7. Liu, Y. Y.; Wang, Y.; Walsh, T. R.; Yi, L. X.; Zhang, R.; Spencer, J.; Doi, Y.; Tian, G.; Dong, B.; Huang, X.; Yu, L. F.; Gu, D.; Ren, H.; Chen, X.; Lv, L.; He, D.; Zhou, H.; Liang, Z.; Liu, J. H.; Shen, J., Emergence of plasmid mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* 2016, 16, (2), 161-8.
8. Xavier, B. B.; Lammens, C.; Ruhhal, R.; Kumar-Singh, S.; Butaye, P.; Goossens, H.; Malhotra-Kumar, S., Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. *Euro Surveill* 2016, 21, (27).
9. Yin, W.; Li, H.; Shen, Y.; Liu, Z.; Wang, S.; Shen, Z.; Zhang, R.; Walsh, T. R.; Shen, J.; Wang, Y., Novel Plasmid-Mediated Colistin Resistance Gene *mcr-3* in *Escherichia coli*. *MBio* 2017, 8, (3).
10. Carattoli, A.; Villa, L.; Feudi, C.; Curcio, L.; Orsini, S.; Luppi, A.; Pezzotti, G.; Magistrali, C. F., Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. *Euro Surveill* 2017, 22, (31).
11. Borowiak, M.; Fischer, J.; Hammerl, J. A.; Hendriksen, R. S.; Szabo, I.; Malorny, B., Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B. *J Antimicrob Chemother* 2017, 72, (12), 3317-3324.
12. Yang, Y.Q.; Li Y.X.; Lei, C.W.; Zhang, A.Y.; Wang, H.N., Novel plasmid-mediated colistin resistance gene *mcr-7.1* in *Klebsiella pneumoniae*. *J Antimicrob Chemother*. 2018, 73, (7), 1791-1795.
13. Wang, X.; Wang, Y.; Zhou, Y.; Li, J.; Yin, W.; Wang, S.; Zhang, S.; Shen, J.; Shen, Z.; Wang, Y., Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. *Emerg Microbes Infect.* 2018, 7, (1):122. doi: 10.1038/s41426-018-0124-z.

**Figure 1.** S1-nuclease restriction and pulsed-field gel electrophoresis (PFGE): plasmids of donor *E. coli* isolates from flies and their transconjugants.



Lane	Sample no. and site	Isolate no.	ESBL	Other $\beta$ -lactamases	PMQR gene	Phylogenetic group	PFGE-type (clone)	Plasmid sizes
1	20 H	752/17	CTX-M-1	-	-	A	E1	80kb, 90kb
2	-	752/17 K1	CTX-M-1	-	-	A	-	80kb
3	32 H	754/17	CTX-M-1	TEM	-	A	E3	90kb, 100kb, 160kb
4	-	754/17 K1	CTX-M-1	TEM	-	A	-	160kb
5	61 Z	758/17	CTX-M-1	-	-	A	E5	90kb
6	-	758/17 K1	CTX-M-1	-	-	A	-	90kb
7	62 Z	759/17	CTX-M-3	-	-	D	E6	80kb
8	-	759/17 K1	CTX-M-3	-	-	A	-	80kb
9	79 Z	763/17	CTX-M-1	-	-	D	E9	105kb
10	-	763/17 K1	CTX-M-1	-	-	A	-	105kb
11	157 RB	768/17	CTX-M-1	LAP	<i>qnrS1</i>	A	E14	50kb, 90kb
12	-	768/17 K1	CTX-M-1	LAP	<i>qnrS1</i>	A	-	50kb
13	31 H	753/17	SHV-12	-	<i>qnrS1</i>	A	E2	35kb
14	-	753/17 K1	SHV-12	-	<i>qnrS1</i>	A	-	35kb
15	48 H	755/17	SHV-12	-	<i>qnrS1</i>	A	E2	35kb
16	-	755/17 K1	SHV-12	-	<i>qnrS1</i>	A	-	35kb

Sites: H = hospital, Z = zoo, RA = residential area A, RB = residential area B; PMQR = plasmid mediated quinolone resistance; K1 = transconjugant *E. coli* J53 Azi<sup>R</sup>; Lane M = *Salmonella* Braenderup H9812 (molecular marker strain, XbaI-restricted).