Transparency declarations

None to declare.

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Inoculation of mice with avian *bla*$_{CTX-M-1}$- or *bla*$_{CMY-2}$-carrying *Escherichia coli* strains does not lead to long-term colonization

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Sir,

ESBL/plasmid-located AmpC (pAmpC)-producing Enterobacteriaceae have been frequently reported in broilers and therefore have been considered as a reservoir for ESBL/pAmpC-producing bacteria and resistance genes for humans. Transmission from broilers to humans could occur by direct contact, via the environment or through the food chain. Contact with broilers has been identified as a risk factor for being ESBL/pAmpC positive for individuals living and/or working on broiler farms and *Escherichia coli* isolates of the same MLST carrying identical ESBL/pAmpC genes on the same plasmid families have been identified in broilers and humans indicating transmission.[1] It has been postulated that humans acquire ESBL/pAmpC-producing bacteria by eating chicken meat, because chicken meat has been shown to be contaminated with *E. coli* strains containing ESBL genes similar to those found in patients.[2,3] Moreover, in Dutch studies, all conventional broiler meat samples and 84% of organic broiler meat samples were contaminated with ESBL-producing microorganisms. Median loads of these microorganisms were 80 cfu/25 g (range <20–1360) in conventional and <20 cfu/25 g (range 0–260) in organic broiler meat samples.[4] The presence of resistant bacteria on meat as such is no proof that this poses a risk to consumers. To date, it is unknown if the consumption of contaminated meat leads to colonization of the human gut and, if so, whether this is dose dependent and what amount of ESBL/AmpC-producing bacteria would be needed to achieve this. This information is important in order to establish microbiological limits for the number of ESBL/pAmpC-producing bacteria on broiler meat. The risk of exposure of humans to third-generation cephalosporin-resistant *E. coli* has been estimated using a farm-to-fork model. The results showed that the probability to be exposed to >10, 100, 1000 or 10 000 cfu by a serving with broiler meat was 7.0%, 3.3%, 1.5% and 0.39%, respectively.[5]

The objective of the present study was to investigate whether two avian ESBL/pAmpC-producing *E. coli* strains administered at a low or high dose can colonize a mammalian gut system using a murine model. This study was approved by the ethics committee for animal experiments of the National Institute for Public Health

Table 1. Number of ESBL/pAmpC-positive mice and an estimation of the cfu/g of faeces in the four treatment groups

	Day									
	0	1	2	3	4	7	14	21	28	35
CMY-2 (low dose)	0	1 NC	1 4.8×10^3	1 9.5×10^3	1 9.5×10^3	1 3.0×10^4	0	0	0	0
CMY-2 (high dose)	0	3 NC	1 5.2×10^4	1 4.8×10^4	1 6.1×10^5	2 4.8×10^3	0	0	0	0
CTX-M-1 (low dose)	0	0	0	0	0	0	0	0	0	0
CTX-M-1 (high dose)	0	4 NC	1 4.8×10^3	0	0	1 NC	0	0	0	0
Total	0/16 0.0%	8/16 50.0%	3/16 18.8%	2/16 12.5%	2/16 12.5%	4/16 25.0%	0/16 0.0%	0/16 0.0%	0/16 0.0%	0/16 0.0%

NC, not countable.
CMY-2 represents an *E. coli* ST57, belonging to phylogenetic group D2 with an IncK plasmid that contains bla_{CMY-2}.[1]
CTX-M-1 represents an *E. coli* ST2223, belonging to phylogenetic group A0 with an IncI1 plasmid that contains $bla_{CTX-M-1}$.[1]

and the Environment. Specific pathogen-free, 8-week-old, female mice ($n=40$) were housed in four treatment groups of 8 mice and one uninfected control group of 8 mice. The mice were not treated with antimicrobials prior to or during the experiment. Four animals from each treatment group remained uninfected to study transmission while the other four were inoculated orally with either a high ($\pm10^6$ cfu) or a low ($\pm10^2$ cfu) dose of avian ESBL/pAmpC-producing *E. coli* from a previous study.[1] In two treatment groups (either high dose or low dose), an avian *E. coli* ST2223 isolate carrying $bla_{CTX-M-1}$ on an IncI1 plasmid was used. In the two other groups, *E. coli* ST57 isolated from broiler faeces carrying bla_{CMY-2} on an IncK plasmid was used. The eight uninfected mice from the control group were inoculated with adjuvant. Faeces from individual animals were collected on days 0, 1, 2, 3, 4, 7, 14, 21, 28 and 35 by housing each mouse individually until defecation. ESBL/pAmpC-producing *E. coli* were isolated using selective enrichment and selective plates as described previously.[1] The cfu were estimated by counting colonies on selective plates after serial dilution. β-Lactamase genes were identified by PCR and sequencing.[1] *E. coli* isolates were confirmed as the inoculation strain by MLST as described previously.[1]

All animals were negative for ESBL/AmpC-producing bacteria before the start of the experiment (day 0). ESBL/pAmpC-producing *E. coli* were recovered from the faeces of 8/16 infected mice on day 1, 3/16 infected mice on day 2, 2/16 infected mice on days 3 and 4 and 4/16 infected mice on day 7 (Table 1). ESBL/pAmpC-producing *E. coli* were not detected after day 7 in any of the mice. The cfu could be counted on nine occasions and cfu/g of faeces ranged from 4.8×10^3 to 6.1×10^5. All uninfected control mice remained negative throughout the experiment. Transmission to mice in contact with inoculated mice was not demonstrated, nor was transfer of plasmids to other *E. coli* observed. In all positive mice, the inoculation strain was recovered and no cross-contamination was detected. In conclusion, infection of mice with high or low oral doses of two avian ESBL/pAmpC-producing *E. coli* strains did not lead to long-term colonization of the gut. Although humans in the community are exposed to broiler meat contaminated with ESBL/pAmpC-producing *E. coli*, further studies are needed to elucidate the risk of its consumption.

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Transparency declarations

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