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mcr-1-like detection in commensal *Escherichia coli* and *Salmonella* spp. from food-producing animals at slaughter in Europe

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ABSTRACT

We evaluate here the presence of the *mcr-1*-like and *mcr-2* genes in *Escherichia coli* and *Salmonella* spp. isolated from healthy food-producing animals at slaughter between 2002 and 2014 in Europe.

Isolates were retrieved from cattle, pig and chicken from 11 European countries of production. The susceptibility to colistin and antibiotics used in human medicine was determined by agar dilution. Colistin-resistant isolates were PCR-screened for *mcr* genes. *mcr*-positive isolates were typed by Pulsed-Field Gel Electrophoresis (PFGE) and Multi-Locus Sequence Typing.

Among the 10,206 *E. coli* and 1774 *Salmonella* spp. isolated from cattle, pigs and chickens, 148 *E. coli* and 92 *Salmonella* spp. isolates were resistant to colistin. We found *mcr-1*-like gene in 68 (0.7%) *E. coli* and 2 (0.1%) *Salmonella* isolates whereas none of the isolates tested positive for *mcr-2*. MCR-1-like-positive *E. coli* were isolated from 2008 to 2014 in chicken (n = 44, 1.2%) and pigs (n = 24, 0.7%). The presence of *mcr-1*-like varied from 0 to 4.0% depending on the year and the animal species. *mcr-1*-like-positive isolates came from animals originating from Germany (n = 38), Spain (n = 23), The Netherlands (n = 5), and France (n = 4). They were distributed in 63 different PFGE types and 37 different STs, with ST10 being the most prevalent. The two *mcr-1*-like-positive *Salmonella* spp. were isolated from France and Germany from a pig and a chicken, respectively.

mcr-1-like gene is present in food-producing animals at slaughter in European countries with the highest occurrence in chickens. The high clonal diversity of *E. coli* underlines the evidence for horizontal transfer of *mcr-1*-like genes.

1. Introduction

Colistin is an antibiotic of last resort for the treatment of human infections due to clinically resistant Gram-negative bacteria such as carbapenem-resistant Enterobacteriaceae (Tzouvelekis et al., 2012). Until recently, resistance to colistin was restricted to chromosomal mutations (Landman et al., 2008). The situation changed in 2015 with the discovery of the plasmid-mediated colistin resistance mechanism MCR-1 described in bacteria isolated in animals, raw meats and in-patients in China (Liu et al., 2016). Since then, the *mcr-1* gene has been reported worldwide in humans, animals and environmental samples (Schwarz and Johnson, 2016). In 2016, a second plasmid-borne gene encoding the colistin resistance mechanism MCR-2 has been isolated from porcine and bovine *Escherichia coli* in Belgium (Xavier et al., 2016). Even more recently, two novel mobile colistin resistance genes, *mcr-3* and *mcr-4*, were discovered from porcine *E. coli* in China, Spain and Belgium (Carattoli et al., 2017; Yin et al., 2017).

Colistin is used for therapeutic purposes in food-producing animals (Pardon et al., 2012) (http://www.ema.europa.eu/ema). Little is known about the prevalence of *mcr* genes in the gut flora of healthy food-producing animals in Europe. Therefore the assessment of the occurrence of *mcr-1* in different meat production chains from different countries is essential. Here, we aimed at evaluating the presence of the *mcr-1*-like and *mcr-2*-like genes in a collection of *E. coli* and *Salmonella* spp. isolated from healthy food-producing animals at slaughter between 2002 and 2014 in Europe.

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Table 1
Distribution according to the country of *mcr-1*-like-positive isolates (n = 70) of commensal *E. coli* (n = 68) and *Salmonella* spp. (n = 2) from food animals at slaughter in Europe between 2002 and 2014.

	Belgium	Denmark	France	Germany	Hungary	Ireland	Italy	Poland	Spain	The Netherlands	United Kingdom	Total
Cattle												
N	305	6	574	658	8	216	443	343	0	0	548	3101
N colistin-resistant	0	0	28	1	0	3	0	0	0	0	4	36
N *mcr-1*-like positive	0	0	0	0	0	0	0	0	0	0	0	0
Pigs												
N	100	689	868	756	40	0	0	0	1057	795	258	4563
N colistin-resistant	0	4	9	18	0	0	0	0	47	1	6	85
N *mcr-1*-like positive	0	0	2	0	0	0	0	0	23	0	0	25
Chicken												
N	0	0	684	667	876	0	0	0	746	752	591	4316
N colistin-resistant	0	0	4	58	7	0	0	0	45	5	0	119
N *mcr-1*-like positive	0	0	2	38	0	0	0	0	0	5	0	45
Total												
N	405	695	2126	2081	924	216	443	343	1803	1547	1397	11,980
N colistin-resistant	0	4	41	77	7	3	0	0	92	6	10	240
N *mcr-1*-like positive	0	0	4	38	0	0	0	0	23	5	0	70

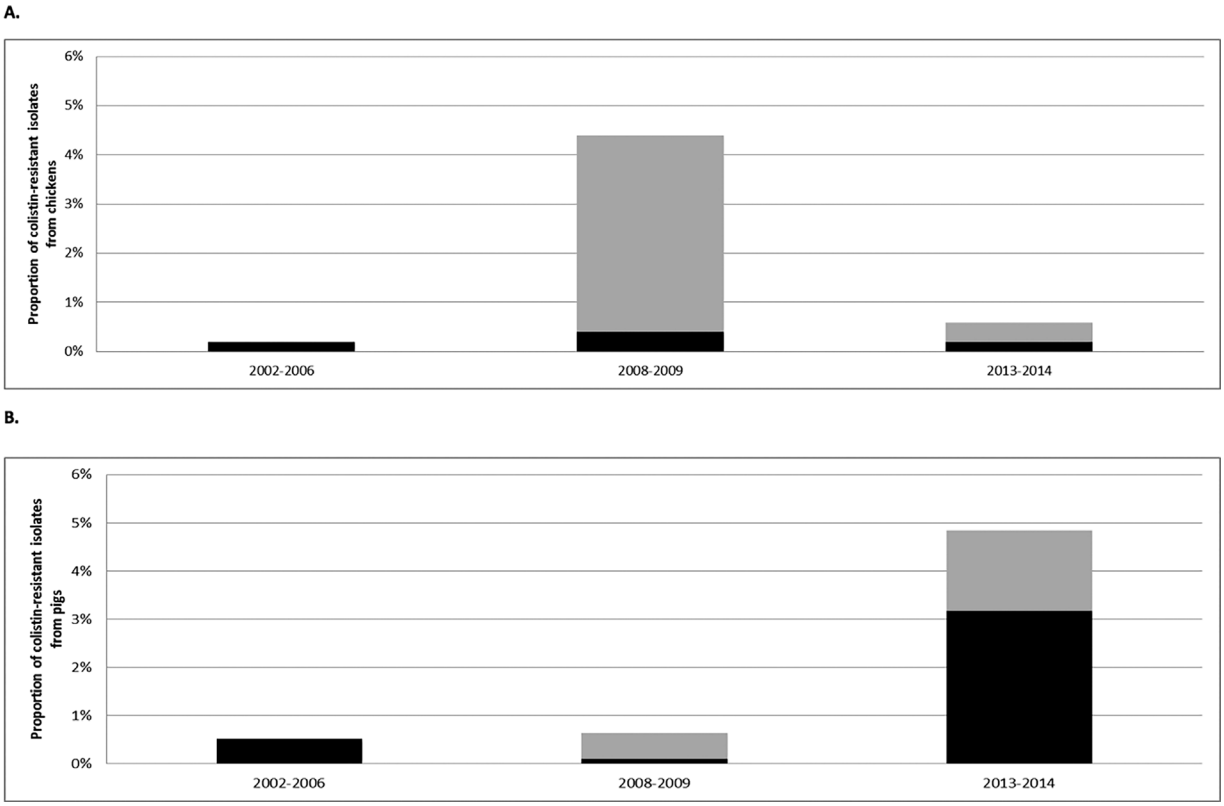

Fig. 1. Proportion of colistin-resistant *mcr-1*-like-positive isolates (grey bars) and proportion of colistin-resistant *mcr-1*-like-negative isolates (black bars) in commensal *E. coli* from chickens (A) and pigs (B) at slaughter in Europe. No *mcr-1*-like-positive isolates were detected in cattle.

2. Methods

2.1. Strain collection

The European Antimicrobial Susceptibility Surveillance in Animals program (EASSA) monitors the antimicrobial susceptibility of zoonotic and commensal bacteria in healthy food-producing animals at slaughter throughout Europe (de Jong et al., 2013). From 1999 to 2014, four EASSA programs have been completed (EASSA-I in 1999–2001, EASSA-II in 2002–2006, EASSA-III in 2008–2009 and EASSA-IV in 2013–2014). The EASSA project includes the major countries of production of cattle, pig and chicken in the EU. Four to six countries were selected per animal species with ≥4 slaughterhouses in each country providing samples. The majority of the slaughterhouses were the same in the different EASSA programs. Colon or caecal isolates were collected from each of the major food-producing animal species (beef cattle, slaughter pigs and broiler chickens) (de Jong et al., 2013). One isolate for each bacterial species was retained from each animal which was randomly selected as being representative of a whole herd. Our study targeted *E. coli* and *Salmonella* spp. with 10,206 *E. coli* and 1774 *Salmonella* spp. isolated from cattle, pigs and chicken between 2002 and 2014. EASSA I collection (1999–2001) was not retained in this study since not tested for colistin susceptibility.