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Colistin resistance was investigated in 1,696 isolates collected from 2007 to 2014 within the frame of the French livestock antimicrobial resistance surveillance programme. The *mcr-1* gene was detected in all commensal *Escherichia coli* isolates with a minimum inhibitory concentration to colistin above the 2 mg/L cut-off value (n=23). In poultry, *mcr-1* prevalence was 5.9% in turkeys and 1.8% in broilers in 2014. In pigs, investigated in 2013, this prevalence did not exceed 0.5%. These findings support that *mcr-1* has spread in French livestock.

We report *mcr-1* prevalence data in commensal *Escherichia coli* isolated from French livestock from 2007 to 2014.

Laboratory investigation

According to the European Union surveillance programme on antimicrobial resistance in zoonotic and commensal bacteria (directive 2003/99/EC) [1], a random sample of faecal (until 2013) or caecal (since 2014) content from the same epidemiological unit (defined as in [2]) of broilers, pigs and turkeys was taken at slaughter houses all over the country, in order to be representative of national productions. The sampling was proportional to the slaughter houses' annual throughputs and was spread over the year. The number of samples collected per animal species and year was calculated to be able to recover at least 170 *E. coli* isolates for each combination of bacterial species and animal production. Isolates were streaked on MacConkey medium, identified and tested for antimicrobial susceptibility by the broth microdilution method (Trek diagnostic systems) using a panel of 14 antimicrobial substances. The minimum inhibitory concentrations (MIC) obtained were compared with the epidemiological cut-off values of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [3]. The DNA of strains with a colistin MIC over 2 mg/L was extracted and the presence of *mcr-1* sought by polymerase chain reaction (PCR) [4].

Colistin resistance and presence of the *mrc-1* gene in isolates

Most (1,427/1,450; 98%) commensal *E. coli* strains isolated and tested from French livestock between 2007 and 2014 were susceptible to colistin (Table).

During the study period however, a total of 23 isolates were resistant to colistin at concentrations above the cut-off value of 2 mg/L, with MICs ranging from 4 to 16 mg/L. Interestingly, each individual *E. coli* isolate from French livestock with a MIC to colistin greater than the cut-off harboured the *mcr-1* gene. From 2011 to 2013, two strains resistant to colistin were isolated from healthy pigs. The prevalence of colistin resistance in broilers was 1.8% in 2014. In turkey production, monitoring commensal *E. coli* became mandatory at European level in 2014 and the prevalence of resistance to colistin was 5.9% that year. Co-resistance patterns were diverse, ranging from one to eight associated mechanism of resistance (data not shown). Nevertheless, in four of the 14 *mcr-1* positive turkey isolates, colistin resistance coincided with simultaneous resistance to ampicillin, quinolones, sulfamethoxazole, tetracycline and trimethoprim (data not shown). One single strain derived from turkeys was also resistant to cefotaxime and carrying the bla_{CMY-2} gene. Plasmid profiling in order to assess the transferability of these *mcr-1* genes from food producing animals to other hosts such as humans is under progress.

Discussion

For decades, colistin has been widely used in veterinary medicine against infections caused by *Enterobacteriaceae* in food-producing animals in Europe [5]. To offset limited data on colistin resistance in European livestock, this antibiotic was added in 2014 to the antimicrobial substances required to be tested under antimicrobial resistance programmes conducted by European Member States (decision 2013/652/EU [2]).

Table

Colistin resistant and mcr-1 positive commensal *Escherichia coli* strains from French livestock, France, 2007–2014

Year	Animals	*E. coli* strains tested for MIC N	*E. coli* strains resistant to colistin N	Proportion of *mcr-1* positive (n) among colistin-resistant *E. coli* strains (N) n/N	Prevalence of *mcr-1* positive *E. coli* strains % (95%CI)
2014	Turkeys	239	14	14/14	5.9 (2.9–8.8)
	Broilers	227	4	4/4	1.8 (0.1–3.5)
2013	Pigs	196	1	1/1	0.5 (0.0–1.5)
	Broiler	193	3	3/3	1.6 (0.0–3.3)
2012	Pigs	194	0	N.a.	N.a.
	Broiler	201	0	N.a.	N.a.
2011	Pigs	200	1	1/1	0.5 (0.0–1.5)
2007	Turkeys	ND[a]	ND[a]	0/246[a]	0 (0.0–1.2)
Total	**All**	**1,450**	**23**	**N.a.[a]**	**N.a.[a]**

CI: confidence interval; MIC: minimum inhibitory concentration; N.a.: not applicable; ND: not determined.

[a] As susceptibility to colistin was not tested in 2007, each isolate obtained in that year was tested for the presence of *mcr-1*.

In spite of this, prior to 2015, the mechanism of resistance to colistin was only known to involve chromosomal mutations, and so its spread was expected to be limited to vertical transmission [6]. In 2015 however, the first plasmid-mediated colistin resistance involving the *mrc-1* gene was discovered in China by Liu et al. [4]. Since, other reports detail retrospective detection of this gene in *E. coli* from animal origin. In Germany, the gene was found in three of the 129 whole-genome sequences of *E. coli* isolated from livestock since 2009 [7]. The *mrc-1* positive strains originated from swine and were sampled in 2010 and 2011. The *mcr-1* gene was also detected in five *E. coli* isolates from chicken meat of European origin imported in Denmark in 2012, 2013 and 2014 [8]. In Belgium, 13 of 105 colistin-resistant *E. coli* isolates collected in 2011 and 2012 from piglets and bovine calves with diarrhoea were positive for *mcr-1* [9]. Also, in France, extended-spectrum beta-lactamase (ESBL)-positive *E. coli* isolated from diarrhoeic bovine calves as early as 2005 were confirmed to be *mcr-1* positive [10] as well as four *Salmonella* isolates from 2012 to 2013 collected within the French agricultural food sector [11]. A number of these findings implicated pathogenic strains, isolated in the context of event-based surveillance networks or programmes.

Prompted by these reports of *mrc-1*-mediated colistin resistance, we investigated the prevalence of *mcr-1* in non-pathogenic *E. coli* isolated through the official European surveillance programme on antimicrobial resistance in French livestock. This programme is designed to be comparable between Member States but its power to detect emergent resistance is likely to be limited. In fact, after three years of continuous monitoring, starting from an initial theoretical point of 0.1% of resistant isolates, this programme cannot detect any changes if the overall increase is lower than 2% per year [2]. The fact that *mcr-1* emergence is detected through this surveillance programme supports the idea of a rapid spread of plasmid-mediated colistin resistance in French livestock.

The presence of co-resistances in strains harbouring the *mcr-1* gene could have contributed to select and enhance the rapid dissemination of the plasmid-mediated resistance to colistin jointly with antibiotic pressure by other antimicrobial use in food producing animals.

The dissemination of *mcr-1* in French livestock, either in a pathogenic or healthy context, raises the question of colistin use in animals. Colistin use should be now revisited in a double perspective: first, in a veterinary medicine perspective, that might suddenly start to face treatment failures in animal digestive disorders such as colibacillosis or salmonellosis; and second, in a human medicine perspective, in order to maintain the efficacy of a last-resort therapeutic option to counteract multidrug-resistant bacterial infections [5].

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Conflict of interest

None declared.

Authors' contributions

APG designed the study, analysed and interpreted data, drafted and coordinated the manuscript elaboration, MB analysed the data and contributed to the manuscript, PH, KD, PL, CP produced phenotypic and molecular data, CS contributed to the manuscript, PS contributed to the manuscript and given scientific advice.

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