

First identification of *mcr-1/mcr-2* genes in the fecal microbiota of Canadian commercial pigs during the growing and finishing period

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Colistin is considered one of the last-resort antibiotics used for the treatment of infections caused by multidrug-resistant Gram-negative bacteria (MDR-GNB) in humans.¹ This antibiotic is classified by the World Health Organization, among the category of the Highest Priority Critically Important Antimicrobials for human medicine.² In this regard, the global dissemination of plasmid-mediated colistin resistance, conferred by the mobile colistin resistance (*mcr*) genes, has raised serious concern among physicians and scientists worldwide.² On the other hand, colistin has been widely used in food animals in many countries either for disease therapy, prophylaxis or for growth promotion.³ Many studies reported a high prevalence of *mcr* genes on bacterial isolates from animals' origin compared to humans, and consequently animals, particularly pig production, were pointed out as the greatest cause of bacterial colistin resistance spread.¹ In pigs, *mcr* genes were isolated mainly from colistin resistant *E. coli*, *Salmonella* and *Klebsiella* isolates, with *mcr-1* gene being the most frequently identified gene among the eight types of *mcr* genes currently described.¹ Among other Gram-negative bacteria (GNB) of pig origin, *mcr* genes were identified in *Moraxella* spp.,⁴ and in *Aeromonas hydrophila*,⁵ enforcing the interest to consider the *mcr* presence at the microbiota level. In Canada, although colistin is not approved for use in veterinary medicine, it was sometimes used in pigs according to special precautions for the oral treatment of digestive infections caused by GNB.¹ Nevertheless, *mcr-1* gene has been identified in *E. coli* isolated from humans, as well as from lean ground beef in Canada.⁶ However, to the best of our knowledge, *mcr* genes have never been identified in animals at the farm level in Canada. Here we report the screening of fecal samples obtained from one Canadian commercial pig farm, to determine the prevalence over the growing period of *mcr-1* and *mcr-2* genes in the fecal microbiota of pigs. Colistin has never been used in this farm. However, tiamulin and chlortetracycline were used in the post-weaning period and salinomycin as well as narasin were used during the growing phase. The present study was primarily designed to investigate the relationship between gut microbiota composition and some welfare problems in pigs (e.g., tail biting). Fecal samples have been collected between January 2017 and April 2017, from one commercial pig farm located in the province of Quebec (Canada). Pigs were sampled 5 times during the study period. In fact, fresh fecal material was collected directly from each animal's rectum using disposable gloves, and immediately 1 g of each sample was placed in liquid nitrogen. At the laboratory, these samples were stored at -80°C until DNA extraction. In the current study, fecal samples that have

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been obtained from 62, 60, 62, 33, and 44 pigs, which correspond to the sampling time; I, II, III, IV and V respectively (Table 1), were used to identify *mcr-1* and *mcr-2* genes. Total genomic DNA was extracted using a standard Phenol-Chloroform method. All DNA extracts were screened by PCR for the presence of *mcr-1* and *mcr-2* genes, using primers and conditions as previously described.⁷ Positive samples for *mcr-1* and *mcr-2* genes were confirmed by the Sanger sequencing of the amplified fragment. The *mcr-1* gene has been identified in all sampling dates. However, *mcr-2* gene was detected in three of the five sampling dates (Table 1). Only one animal harbor in its fecal microbiota both *mcr-1* gene and *mcr-2* gene, however this finding was transitory, since this result was observed only at the first sampling time. It is noteworthy that 11.4% of pigs were sent to the slaughterhouse with *mcr-1* gene in their microbiota, which could represent a threat for consumers in terms of colistin-resistance genes transfer. Our results corroborate the study of Meinersmann et al who reported the identification of *mcr-1* gene in the cecal contents of pigs in the United States, even in the absence of evidence of colistin use in pigs in this country.⁸ However, the Canadian Integrated Program for Antimicrobial Resistance Surveillance targets only the antimicrobial resistance profile of *E. coli* and *Salmonella* at pig farms, which can explain in part why the *mcr* genes have never been identified at the farm level in Canada. Our results highlight the need to expand monitoring *mcr* genes and colistin resistance to other *Enterobacteriaceae* species than those routinely analyzed. In our study, the bacterial genus harboring the *mcr-1* and/or the *mcr-2* genes was not identified. However, we unequivocally confirm the presence of these genes in the microbiota of pigs in a conventional facility. It should be stressed here that *mcr-1* and *mcr-2* genes have been identified in the fecal microbiota of pigs that have never been treated with colistin. Such presence could be related to the possible contamination of feed by bacteria harboring these genes. In fact, *mcr-1* gene has already been isolated from colistin-resistant *E. coli* recovered from animal feed.⁹ On the other hand, *mcr-1* gene was identified in 60% of air samples from finishing swine barns in Canada.¹⁰ Thereby, swine confinement buildings could be also a source of contamination for pigs. Moreover, based on known co-localization of *mcr* genes with other antimicrobial genes within the same mobile genetic elements, selective pressure imposed by other antimicrobials could favor the selection of *mcr* genes, even in the absence of colistin use. Indeed, it was reported that a colistin-resistant *E. coli* strain derived from animal origin harbored on the same plasmid, in addition to *mcr-1* gene, several resistance-encoding genes such as; trimethoprim (*dhfrAI*), tetracycline (*tetA*),

Table 1 Detection of *mcr-1* and *mcr-2* genes in the fecal microbiota in a Canadian commercial pigs farm during the growing and finishing period, 2017

Sampling number	Sampling time from the beginning of the growing period	Number of fecal samples investigated	Number of <i>mcr-1</i> positives samples/(in %)	Number of <i>mcr-2</i> positives samples/(in %)	Number of <i>mcr-1</i> and <i>mcr-2</i> positives samples/(in %)
I	34 days	62	6 (9.7)	6 (9.7) ^b	1 (1.6)
II	47 days	60	15 (25.0) ^a	0 (0.0)	0 (0.0)
III	61 days	62	1 (1.6)	4 (6.5)	0 (0.0)
IV	75 days	33	3 (9.1)	12 (36.4) ^b	0 (0.0)
V	109 days (one day before sending animals to the slaughterhouse)	44	5 (11.4) ^a	0 (0.0)	0 (0.0)

Notes: ^aFecal samples derived from the same animals (N=3), were positive for *mcr-1* gene at I and III. ^bFecal samples derived from the same animals (N=2), were positive for *mcr-2* gene at I and IV.

aminoglycoside (*aadA1*, *aph(6)-Id* or *strA*, and *aph(3'')-Ib/strB*), and sulphonamide (*sul1*) antibiotics.¹¹ In our study, the use of chlortetracycline could be associated with a selective pressure on *mcr* genes. However, to the best of our knowledge, neither cross-resistance between colistin and coccidiostat ionophore drugs (e.g., salinomycin, narasin), nor between colistin and tiamulin has been reported in the scientific literature.

Further studies are needed to determine the role of the selection pressure by other antimicrobials as well as pig's environment and feed in the dissemination of colistin resistance genes in pigs.

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Disclosure

The authors report no conflicts of interest in this work.

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