High prevalence of MCR-1-producing Escherichia coli and Klebsiella pneumoniae in pigs, Portugal

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Background: The mcr-1 gene encoding a phosphoethanolamine transferase has been recently identified as a source of acquired resistance to polymyxins in Escherichia coli. It has been occasionally identified in other species such as Enterobacter cloacae and Klebsiella pneumoniae. It is now admitted that the mcr-1 gene has very likely emerged first in animals due to a high selective pressure with colistin in veterinary medicine since the mid 1960’s. Our study aimed to prospectively analyze the prevalence and the occurrence of the mcr-1 gene among Enterobacteriaceae recovered from two pig farms in Portugal.

Material/methods: One-hundred fecal samples from two different Portuguese pig farms were screened for polymyxin-resistant Enterobacteriaceae using the Superpolymyxin® selective plates. Isolates were confirmed to be resistant to colistin by using the biochemical Rapid Polymyxin NP test. Susceptibility testing was evaluated by broth microdilution for colistin and by disk diffusion for other antibiotics.
Screening of the *mcr-1* gene and other resistant determinants was performed by PCR amplification followed by sequencing. PCR-based replicon typing (PBRT) was realized with the PBRT kit (Diatheva®). Clonality and phylogeny assays were determined by PFGE analysis, by MLST, and by the Clermont method identifying *E. coli* phylogroups.

**Results:** Ninety-eight isolates (18 *K. pneumoniae* and 80 *E. coli*) being resistant to colistin were recovered. Noteworthy, they all carried the *mcr-1* gene. Among the *E. coli mcr-1* positive strains, 7 co-produced a TEM-like extended-spectrum ß-lactamase. All *K. pneumoniae* isolates belonged to Sequence Type ST45 and all *E. coli* belonged to the B1, A and F phylogenetic groups. Twenty-nine different *E. coli* clones were identified belonging to twenty-six different ST. Insertion sequence ISApl1 was identified upstream of the *mcr-1* gene in all isolates from farm 1, but absent in all isolates of farm 2. PBRT showed that the *mcr-1* gene was carried either onto IncHI2 or IncP plasmids.

**Conclusions:** This study showed a wide dissemination of MCR-1 in two different pig farms in Portugal, further highlighting the wide diffusion of that colistin resistance determinant in veterinary medicine. Furthermore, we showed here the worrying dissemination of *mcr-1* not only in *E. coli* but also significantly in *K. pneumoniae*.