Journal of Antimicrobial Chemotherapy

J Antimicrob Chemother 2016; **71**: 2326–2361
doi:10.1093/jac/dkw074
Advance Access publication 29 March 2016

Emerging plasmid-encoded colistin resistance: the animal world as the culprit?

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Sir,

A recent study from China identified transferable polymyxin resistance in Gram-negative bacilli from human and animal isolates.[1] Although polymyxins, including colistin and polymyxin B, were among the very few antibiotics for which no transferable resistance had been identified, we are now facing a novel threat with the identification of a plasmid-encoded resistance mechanism. Colistin is an old drug that was first introduced in 1959 but remained on the shelf for many years due to renal and neurotoxicity.[2] However, we are experiencing a renewed interest in this drug due to the rapid emergence of MDR Gram-negative organisms.

The plasmid-borne *mcr*-1 gene encodes a phosphoethanolamine transferase that mediates addition of phosphoethanolamine to the lipid A moiety of the lipopolysaccharide, consequently conferring resistance to polymyxins.[1] This gene has thus far mainly been found in *Escherichia coli*, and to a lesser extent in *Klebsiella* spp. and *Salmonella* spp.

Very shortly after the original report, other studies reported identification of the *mcr*-1 gene in human *E. coli* isolates in many different countries, including in Algeria, Denmark, France, Germany, Laos, the Netherlands, Malaysia, Nigeria, Switzerland and Thailand.[3–5] It was also identified from imported food products in Denmark (meat) and Switzerland (vegetables).[6,7] In addition, plasmid-encoded carbapenem and colistin resistance may be co-associated in *E. coli*.[3,5,8] In addition, positive *Salmonella enterica* isolates of different serotypes were identified from food samples in Portugal in 2011, and France in 2012 and 2013.[9,10] Finally, an epidemiological survey conducted in France on a collection of ESBL-producing *E. coli* isolates ($n=517$) recovered from the faeces of diarrheic veal calves on farms from 2005 to 2014 showed a very high rate of MCR-1-positive isolates (20.5%).[11]

Taken together, these findings indicate that: (i) the spread of *mcr-1* is not recent, as it has already occurred worldwide, probably due to its location on conjugative plasmids; and (ii) *E. coli* is so far the main reservoir of this resistance trait among human, animal and environmental isolates. This is a source of concern since *E. coli* isolates are easily exchanged from the environment to humans in which they may remain as a commensal in the gut flora; in addition, *E. coli* is the number one pathogen for humans.

MCR-1 is one of the few and clear examples of the animal origin of a resistance trait that may later hit the entire human health system, along with the examples of some MRSA clones (such as CC398), the serotype O104:H4 CTX-M-15 ESBL-producing enteroaggregative *E. coli* and ESBL-producing *Salmonella* spp. strains.

The animal origin of MCR-1 is sustained by: (i) the number of reports of animal isolates expressing *mcr-1*, which is already high; and (ii) heavy usage of colistin in veterinary medicine. By standardizing the sales of antimicrobials in relation to the total weight of animals 'at risk' undergoing treatment across Europe in 2011, it was estimated that polymyxins are the fifth most sold group of antimicrobials (7%).[12] In Europe, colistin and polymyxin B are used for treating infections caused by Enterobacteriaceae in rabbits, broilers, veal, beef cattle, dairy cattle and (primarily) pigs. In addition, in other parts of the world, polymyxins are used as growth promoters, a usage that has been banned in Europe since 2006. The animal origin of MCR-1 is also sustained by the genetics associated with the *mcr-1* gene. When investigating the *mcr-1*-positive *E. coli* KRI recovered from the urine of a community patient in Switzerland,[3] an insertion sequence (IS*Apl1*) was identified upstream of the *mcr-1* gene that was 100% identical to one identified in *Pasteurella multocida*, which is a common animal pathogen, in particular for pigs. In addition, this same *E. coli* co-expressed the broad-spectrum β-lactamase gene bla_{CMY-2}, and the florfenicol resistance gene *floR*, both genes that are widely disseminated in animal isolates, with florfenicol being used in veterinary medicine only.

It seems very likely that the occurrence of polymyxin resistance in animal isolates has been underestimated and unrecognized for years, since the determination of polymyxin susceptibility is difficult. Disc diffusion and Etest are not reliable techniques, and broth microdilution is the gold standard technique but it is cumbersome and not used on a regular basis, in particular in veterinary medicine. Therefore, there is an urgent need for rapid diagnostic tests for polymyxin resistance in Enterobacteriaceae.

The impact of the use of polymyxins in agriculture was not seriously taken into account as long as there was no critical need for colistin in human medicine. However, times have changed and a co-ordinated re-evaluation of polymyxin usage in agriculture is urgently needed to prevent selection in veterinary medicine of polymyxin-resistant isolates that might subsequently be transferred to humans. Similarly, using polymyxins in selective digestive decontamination to prevent the spread of MDR bacteria in hospitals should be discouraged. Nevertheless, a decision to ban polymyxins in agriculture would be far from simple and might be a matter of balancing risk against benefits.

In a recent review debating the use of colistin-containing products within the European Union and European Economic Area, Catry *et al.*[12] concluded by stating 'Should colistin resistance determinants be found on mobile genetic elements in bacteria of concern from human or animal origin, or should a clonal expansion of pathogenic polymyxin-resistant bacteria take place, further risk profiling would be required'. Here we are, unfortunately!

Funding

This work was funded by the University of Fribourg, Switzerland.

Transparency declarations

None to declare.

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J Antimicrob Chemother 2016
doi:10.1093/jac/dkw122
Advance Access publication 13 April 2016

Colistin resistance gene *mcr*-1 in *Escherichia coli* isolates from humans and retail meats, Taiwan

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Sir,

The gene encoding plasmid-mediated colistin resistance, *mcr*-1, was recently reported for the first time by Liu *et al.*[1] The present study investigated the prevalence of *mcr*-1 in *Escherichia coli* and their molecular characteristics from the biennial Taiwan Surveillance of Antimicrobial Resistance (TSAR) programme[2] and those isolated from retail meats purchased from traditional and supermarkets in Taiwan.

A total of 4589 non-duplicate clinical *E. coli* isolates, including 1136, 1752 and 1701 from 2010, 2012 and 2014, respectively, were tested for colistin susceptibility by the broth microdilution method using Sensititre panels (Trek Diagnostics, England). Twenty isolates, 2 (0.2%) from 2010, 3 (0.2%) from 2012 and 15 (0.9%) from 2014, had colistin MICs >2 mg/L, among which 1 (0.1%), 2 (0.1%) and 11 (0.6%) isolates from 2010, 2012 and 2014, respectively, were positive for *mcr*-1. The specimen source of the *mcr*-1-positive clinical isolates (Figure 1) included ascites (one), abscess (two), blood (three), sputum (one) and urine (seven). The age of the patients ranged from 39 to 88 years. These isolates were from nine hospitals located in all four regions of Taiwan. Except 4 isolates, which were recovered from patients who had been hospitalized for ≥3 days, the other 10 isolates were recovered from either outpatients or patients who had been hospitalized for ≤1 day.

Eighteen colistin-resistant *E. coli* isolates were also identified from our surveillance of antimicrobial-resistant bacteria in retail meats, which included ground beef, chicken and pork, between 2012 and 2015. All 18 isolates were positive for the *mcr*-1 gene. The sequences of the amplicons were 100% identical to that reported by Liu *et al.*[1] The prevalence of *mcr*-1 among the meat *E. coli* isolates was 1.1% (1/89), 6.6% (6/91) and 8.7% (11/126) in 2012, 2013 and 2015, respectively. Three of the *mcr*-1-positive isolates (M10–M12) were from the same meat sample, but had different antibiograms, pulsotypes and STs (Figure 1). The meat samples from which the *mcr*-1-positive isolates were detected included 1 beef (from 2012), 13 chicken (6 from 2013 and 7 from 2015) and 2 pork (from 2015).