Unexpected complexity of multidrug resistance in the *mcr-1*-harbouring *Escherichia coli*

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Dear Editor,

The discovery that the mobile colistin resistance gene (*mcr-1*) is encoded by plasmids and is prevalent in food animals and human beings worldwide (Hasman et al., 2015; Liu et al., 2015) has challenged greatly our traditional idea that polymyxin (consisting of two isoforms: polymyxin B and polymyxin E (colistin)) acts as an ultimate line of refuge in the clinical treatment against the severe infections by the multidrug-resistant Gram-negative pathogens (Nation et al., 2015; Paterson and Harris, 2015). To make matters worse, the *mcr-1* gene was recently found to be co-localized with other drug resistance genes in the plasmid pKH457-3-BE with an IncP backbone from a bovine isolate in Belgium (Surbi Malhotra-Kumar, 2016), which is far different from the pig-isolated plasmid, pHNSHP45 with an IncI2 backbone (Liu et al., 2015). It raised the possibility that super-bugs with pan-drug resistance might be emerging.

Given the fact that (1) genomic sequences of the *mcr-1*-harbouring plasmids are extremely limited right now, and (2) co-occurrence of MCR-1-mediated colistin resistance with other multidrug resistance remains unclear, we screened a collection of antibiotic-resistant isolates (no., 102) from swine tissues in China. In particular, only 6 of the

16 colistin-resistant isolates (namely WH01, WH02, ..., WH16) are verified to be *mcr-1*-positive *Escherichia coli* (Figure 1A, 1B and S1). They are namely WH03, WH07, WH09, WH12, WH13, and WH15. In contrast, the majority of the isolates we checked is *mcr-1*-negative, but remains appreciable level of the colistin resistance (not shown), implying the possibility that some mystical machineries/vectors claim for this antibiotic resistance. Subsequently, we tested the sensitivity of the six *mcr-1*-positive *E. coli* isolates to a dozen of various antibiotics (Figure 1C and S1). The antibiotics (15 in total) we used here are categorized into eight groups 1: β -lactams antibiotics including ampicillin (AMP), cefotaxime (CTX); 2: quinolone antibiotic such as ciprofloxacin (CIP), norfloxacin (NOR) and levofloxacin (LEV); 3: tetracycline antibiotics (tetracycline (TET) and doxycycline (DOX)); 4: aminoglycoside antibiotics like amikacin (AMK), gentamycin (GEN) and kanamycin (KAN); 5: amino alcohol antibiotic, chloramphenicol (CHL); 6: sulfonamide antibiotic, trimethoprim (TMP); 7: nitrofurantoin antibiotic, macrodantin (NFT); and 8: cationic polypeptide antibiotic, colistin (COL)).

To our surprise, the unexpected complexity of the multidrug resistance was observed in the *mcr-1*-harbouring isolates. First, the *mcr-1*-positive isolate WH13 exhibited the mostly-broad-spectrum antibiotic resistance in that it can be tolerant with nearly all the 15 antibiotics with an exception of amikacin (Figure 1C). Second to the WH13, the *mcr-1*-carrying isolate WH12 are resistant to 13 antibiotics (such as cefotaxime, tetracycline and kanamycin) (Figure

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Figure 1 Complicated profile for the multi-drug resistance in *mcr-1*-positive *Escherichia coli* isolates from the swine lung microbiota. A, 16S-based PCR identification for *Escherichia coli* isolates from swine lung microbiota. B, PCR screening of colistin-resistant *E. coli* strains for the presence of the *mcr-1* gene. The colistin-resistant bacterial strains are namely WH03, WH07, WH09, WH12, WH13, and WH15. The expected size of 16S rDNA is around 1.5 kb, and the PCR amplicon of the *mcr-1* gene in full length is about 1.6 kb. kb, kilo-base pair; M, DNA marker. C, Unexpected diversity in multi-drug resistance profile of the *mcr-1*-harbouring swine *E. coli* isolates. β -lactams antibiotics are in red; quinolone antibiotic is in blue; tetracycline antibiotics are in green; aminoglycoside antibiotics are in purple; amino alcohol antibiotic is in black; Sulfonamide antibiotic is in pink; nitrofurantoin antibiotic is in grey; and polypeptide-like antibiotic is highlighted in bold letters. MIC, minimum inhibitory concentration; AMP, ampicillin; CTX, cefotaxime; CAZ, ceftazidime; GEN, gentamicin; KAN, kanamycin; AMK, amikacin; TET, tetracycline; DOX, doxycycline; CIP, ciprofloxacin; NOR, norfloxacin; LEV, levofloxacin; CHL, chloramphenicol; TMP, trimethoprim; NFT, macrolantoin; and COL, colistin (polymyxin E). /, the value less than the cut-off.

1C). Third, the antibiotic resistance profile of the two isolates (WH03 and WH15) reached 9 and 12 antibiotics (Figure 1C). Finally, both isolates WH07 and WH09 showed the resistance to 7 antibiotics (Figure 1C). Of particular note, all the above colistin-resistant isolates still remain susceptible to the aminoglycoside antibiotics amikacin (Figure 1C). Apart from the strain WH13, the remaining five isolates (6 in total) can be effectively inhibited in the presence of the nitrofurantoin antibiotic, macrolantoin (Figure 1C).

Our data provided a glimpse of complicated situation for multidrug resistance in the *mcr-1*-harbouring isolates with the origin of swine lung, suggesting the possibility that diversified *mcr-1*-carring plasmid reservoirs are present in swine lung microbiota. In fact, this speculation has already been verified in the *mcr-1*-harbouring human isolates (Ye et al., 2016). Increasingly-accumulated evidence suggested that the *mcr-1* gene might co-exist with the variants (NDM-5 and NDM-9) of NDM-1 (New Delhi-Metallo- β -lactamase 1), another notorious antidrug gene (Du et al., 2016; Yao et al., 2016). In addition, although it seemed likely that bacterial infections by these *mcr-1*-harbouring pathogens with the multidrug resistance still can be con-

trolled using correct antibiotic-based therapy, it is greatly needed to integrate combined strategies to minimize/control current situation that the multi-drug resistance is increasing worldwide including China.

Compliance and ethics The author(s) declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Figure S1 Gram-staining analyses for determination of *mcr-1*-positive *E. coli* isolates from the swine microbiota.

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