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Letter to the Editor

Detection and genetic characterization of the colistin resistance gene *mcr-3.3* in an *Aeromonas veronii* strain isolated from alligator faeces

Sir,

With the increasing use of colistin in clinical settings and veterinary practice, its antimicrobial efficacy has been challenged by the emergence and worldwide dissemination of the mobile colistin resistance determinants *mcr*. The report of detection of the *mcr-1* gene in wildlife such as migratory birds and Magellanic penguins indicated that they could be a risk factor for dissemination of colistin resistance determinants. The Chinese alligator (*Alligator sinensis*) is a critically endangered crocodile endemic to China, where it is listed as a first-class protected animal at the national level. The Zhejiang Changxing Yangtze alligator protection base is geographically isolated from the external environment and has its own internal circulation waters, maintaining only a certain degree of connection with the external rivers to avoid changes in water quality. Whether organisms harbouring the *mcr* genes have contaminated such an isolated niche remains largely unknown.

Anal swabs were collected from 71 Chinese alligators in November 2018 when the alligators were in their hibernating state. Of these, two (2.82%) were positive for *mcr-3*, whereas all samples were negative for *mcr-1*. However, only one *mcr-3*-positive isolate (HX3), which was identified as *Aeromonas veronii*, was obtained from one sample; an *Aeromonas hydrophila* strain and an *Aeromonas caviae* strain isolated from another *mcr-3*-positive sample were negative for the *mcr-3* gene.

Antimicrobial susceptibility testing was determined by the agar dilution method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline [1]. The *A. veronii* strain HX3 was found to be resistant to piperacillin/tazobactam but susceptible to ceftazidime, cefoperazone/sulbactam, cefepime, aztreonam, amikacin, ciprofloxacin, levofloxacin, tigecycline, trimethoprim/sulfamethoxazole and colistin [minimum inhibitory concentration (MIC) 0.5 mg/L].

Genome sequencing was conducted using the Illumina NextSeq 500 and nanopore MinION sequencer. Hybrid genome assembly with Unicycler v.0.3.1 showed that the *A. veronii* strain HX3 contained one circular chromosome (4 604 603 bp, GenBank: CP040717) and one circular plasmid designated pHX3 (158 215 bp, GenBank: CP040718). pHX3 is an IncA/C plasmid without multidrug-resistant genes [2]. The strain HX3 belonged to a novel sequence type ST568. BLAST (v.2.2.31+) analysis against the Comprehensive Antibiotic Resistance Database (CARD) revealed the presence of multiple antimicrobial resistance genes in the chromosome, including the tetracycline resistance gene *tet(E)*, the β-lactam resistance genes *cphA4* and *ampS* and the mobile colistin resistance gene *mcr-3.3*.

The genetic content surrounding the *mcr-3.3* gene in *A. veronii* HX3 was similar to that in *A. veronii* 172 (Fig. 1). An *mcr-3*-like gene which does not confer colistin resistance was found located 66 bp downstream of the *mcr-3.3* gene in both strains, constituting an identical *mcr-3.3-mcr-3*-like segment [3]. Compared with strain 172, the genetic environment of this segment contains two mobile genetic elements, IS*5* and IS*Aeca1*. A 7102-bp fragment located upstream of the *mcr-3.3-mcr-3*-like segment in strain 172 was replaced by an IS*5* in HX3, and the gene downstream of the IS*5* gene was truncated by the IS*Aeca1* element in HX3. Thus, the genetic context of this segment in the strain HX3 was Δ*HP*-IS*Aeca1*-Δ*HP*-IS*5*-*mcr-3.3-mcr-3*-like-*dgkA-HP*-ΔIS*As1*-*orf*. The *mcr-3.3* gene is known to confer colistin resistance in *Escherichia coli* and *Aeromonas* spp., but the colistin MIC of the host strain is determined by its location (chromosome or plasmid) and copy number [3,4]. *E. coli* and *Aeromonas salmonicida* transformants carrying the plasmid pUC19-*mcr-3.3* exhibited 8- and 64-fold higher MIC values than the transformant carrying pUC19 alone, but the *A. veronii* isolates 172 and HX3 which carried a chromosomal *mcr-3.3* gene were both susceptible to colistin [3]. *A. veronii* is prevalent in the aquatic environment and serves as a potential reservoir of *mcr-3* [4]. Carriage of the *mcr-3.3-mcr-3*-like segment by strains with distinct genetic backgrounds (172: ST512 and HX3: ST568) is likely to be the consequence of transposition or genetic recombination activities of mobile genetic elements; we therefore hypothesize that such activities are mainly responsible for the dissemination of the *mcr-3.3* genes.

Chinese alligators, inhabiting in a relatively isolated base, were fed with fix-point farmed fish, beef and ducklings to activate their intestinal tracks after hibernation. The limited access to the external environment is expected to offer alligators protection against exposure to multidrug-resistant bacteria, including mobilized colistin resistance (MCR)-producing strains. This is the first report of isolation of an *A. veronii* strain containing chromosomal *mcr-3.3* and *mcr-3*-like genes from the faeces of a Chinese alligator in the Zhejiang Changxing Yangtze alligator protection base. Despite carriage of the *mcr-3.3* gene, the strain remains phenotypically susceptible to colistin. The isolation of the *A. veronii*-bearing HX3 strain from the faecal sample of a Chinese alligator expanded the host range of MCR-producing bacteria and indicated that the 'isolated base' is no longer free from *mcr*-bearing organisms. It remains unknown how the *mcr*

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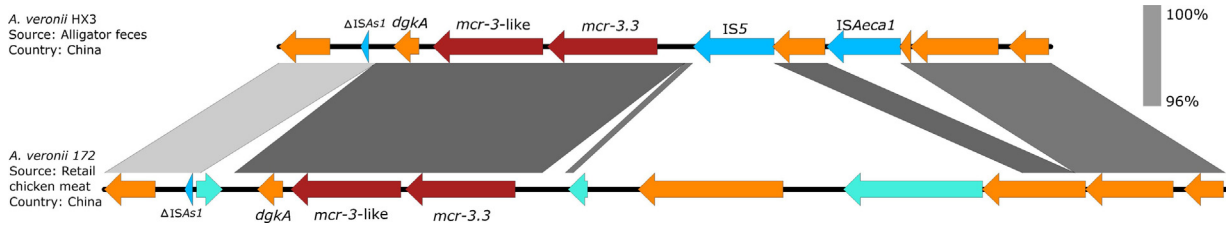

Fig. 1. Genetic environment of the *mcr-3.3* gene in different *Aeromonas* isolates. Red, blue, green and yellow arrows indicate *mcr-3.3* or the *mcr-3*-like gene, insertion sequences, hypothetical proteins and other functional proteins, respectively.

genes contaminated the niche. Future work should be conducted on the presence of *mcr* genes among test food, water and animal handlers. In this study, the discrepancy between the results obtained from direct sample testing and from bacterial isolation is indicative of the existence of unknown sources of resistance termed 'phantom resistome' [5]. It is noteworthy that environmental contamination by resistance genes is underestimated.

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Competing interests

None declared.

Ethical approval

Not required.

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