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

Huang, Zixian; Dong, Ning; Chen, Sheng; Shu, Lingbin; Sun, Qiaoling; Zhou, Hongwei; Wang, Hanyu; Chan, Edward Wai-Chi; Gu, Danxia

Published in:
Journal of Global Antimicrobial Resistance

Published: 01/09/2020

Document Version:
Final Published version, also known as Publisher’s PDF, Publisher’s Final version or Version of Record

License:
CC BY-NC-ND

Publication record in CityU Scholars:
Go to record

Published version (DOI):
10.1016/j.jgar.2020.07.003

Publication details:

Citing this paper
Please note that where the full-text provided on CityU Scholars is the Post-print version (also known as Accepted Author Manuscript, Peer-reviewed or Author Final version), it may differ from the Final Published version. When citing, ensure that you check and use the publisher's definitive version for pagination and other details.

General rights
Copyright for the publications made accessible via the CityU Scholars portal is retained by the author(s) and/or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights. Users may not further distribute the material or use it for any profit-making activity or commercial gain.

Publisher permission
Permission for previously published items are in accordance with publisher's copyright policies sourced from the SHERPA RoMEO database. Links to full text versions (either Published or Post-print) are only available if corresponding publishers allow open access.

Take down policy
Contact lbscholars@cityu.edu.hk if you believe that this document breaches copyright and provide us with details. We will remove access to the work immediately and investigate your claim.
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 widespread 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 crocodilian 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 v0.3.1 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 (v2.2.31+) 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.

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, IS5 and ISAca1. A 7102 bp fragment located upstream of the mcr-3.3-mcr-3-like segment in strain 172 was replaced by an IS5 in HX3, and the gene downstream of the IS5 gene was truncated by the ISAca1 element in HX3. Thus, the genetic context of this segment in the strain HX3 was ΔHP-ISAca1-ΔHP-IS5-mcr-3.3-mcr-3-like-dgA-HP-ΔISAs1-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
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.

Funding

This work was supported by the Basic Research Fund of Shenzhen City [grant number 20170410160041091] and the National Natural Science Foundation of China [grant number 81601815].

Competing interests

None declared.

Ethical approval

Not required.

References