In vivo selection of a multidrug-resistant Aeromonas salmonicida during medicinal leech therapy

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Abstract

We report the selection in a 15-year-old boy of a multidrug-resistant, extended-spectrum β-lactamase (ESBL)-producing Aeromonas salmonicida after medicinal leech therapy that required an antibiotic prophylaxis based on piperacillin/tazobactam and cotrimoxazole. Whole genome sequencing of the strain indeed revealed 13 antibiotic resistance genes, including the ESBL CTX-M-3 and the unusual β-lactamase SCO-1.

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Introduction

Our patient was a healthy 15-year-old boy who experienced traumatic proximal phalange avulsion of his left (nondominant) thumb. The finger was immediately reimplanted with success. Six days later, however, he sought care for acute ischaemia and underwent repeat exploration that identified complete arterial and venous thrombosis. For the venous congestion, medicinal leech therapy (MLT, also referred as hirudotherapy; Fig. 1) was initiated (9 February 2016, referred as day 0; Table 1). Concomitantly, because of surgery and the infectious risk of MLT, piperacillin/tazobactam-based prophylaxis was initiated, and a sample of the leech conservation medium (tap water) was sent at day 8 for culture to the bacteriology laboratory. It yielded Acinetobacter johnsonii, Aeromonas veronii and Moraxella osloensis, all identified by matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (MALDI Biotyper; Bruker Daltonics, Bremen, Germany; database version 3.1) and displaying a wild-type susceptibility to antibiotics (Table 1). MLT was successfully conducted for 14 days while the patient received piperacillin/tazobactam prophylaxis. Various wound swabs were sent for culture, which yielded Stenotrophomonas maltophilia and Aeromonas sp. that remained susceptible to β-lactams. No infectious complication was seen. Prophylaxis with cotrimoxazole was provided for 3 days after MLT ended (from day 13 to day 15). At day 26, a multidrug-resistant Aeromonas salmonicida (named ASG1) was recovered from the wound after culture on blood-supplemented agar plate under aerobic conditions at 35°C and identification by MALDI-TOF MS. The phenotypic traits of ASG1 are shown in the Supplementary Table S1. Of note, no sign of surgical site infection was observed, and the presence of ASG1 was considered to be colonization. Accordingly, no antibiotic treatment was initiated. Another sample of the wound taken at day 35 yielded S. maltophilia but not A. salmonicida (Table 1).

The strain ASG1 displayed a phenotype compatible with the production of an extended-spectrum β-lactamase (ESBL) according to the double-disk synergy test and was resistant to all the tested β-lactams except imipenem (minimal inhibitory concentration (MIC) 0.38 mg/L) and meropenem (MIC 0.5 mg/L). Moreover, the strain was resistant to ciprofloxacin, gentamicin and cotrimoxazole, while it was susceptible to amikacin, fosfomycin, tigecycline and colistin (MIC 0.25 mg/L). Because of this atypical resistance profile, the genome of the strain was sequenced. Patient consent for reporting the case was obtained on 2 November 2016.

Genome Analysis

Genomic DNA was extracted using the MagCore Genomic DNA Tissue Kit (RBC Bioscience, New Taipei City, Taiwan) and was sent to Fasteris (Plan-les-Ouates, Switzerland) for sequencing. The library was prepared using the Nextera XT
DNA Sample Preparation Kit according to the Illumina (San Diego, CA, USA) instructions, and was sequenced on an Illumina MiSeq with 250 bp paired-end reads. The Trimmomatic package [1] was used to remove bases that corresponded to the standard Illumina adapters. A total of 208 982 cleaned reads were obtained (sequence read archive no. PRJNA377399). The reads were assembled with IDBA-UD [2], yielding 150 contigs (the largest contig being 215 626 bp, N50 74 kbp) for a total length of 4 971 056 bp. The median depth of sequencing was 16×. Using JSpeciesWS [3], the average nucleotidic identity (ANI) with *A. salmonicida* type strain ATCC 33658 was 97.0% (range of ANI with other *A. salmonicida* genomes 96.7–97.2%, Supplementary Table S2). The mean ANI with other *Aeromonas* species for which genomes are available was 83.8% (range 76.8–90.4%, Supplementary Table S2). The alignment of the 16S rRNA sequence of ASG1 together with those of other *Aeromonas* species showed that ASG1 clustered with *A. salmonicida* (Fig. 2). The genes were predicted and annotated with PROKKA [6]. We aligned the amino acid sequences of the topoisomerases (*GyrA*, *GyrB*, *ParC* and *ParE*) of ASG1 with those of the reference strain A449 and found the Ser83Ile (*GyrA*) and the Glu93Lys (*ParC*) mutations that likely conferred the ciprofloxacin resistance observed in ASG1. The antibiotic resistance determinants (ARDs) were searched using BLASTP [7] using the ResFinder [8] and ResFinderFG (https://cge.cbs.dtu.dk/services/ResFinderFG/) databases and a cutoff of 80% amino acid identity over 80% of the reference sequence. We found 13 ARDs. Aside from the intrinsic FOX, CphA and OXA-12-like β-lactamases found in *Aeromonas* spp. [9], we identified the CTX-M-3 ESBL, TEM-1 and—more surprisingly—the SCO-1 inhibitor-resistant class A β-lactamase, all of which, taken together, suggest an increased potential for resistance to β-lactam antibiotics in this *Aeromonas* species.

**TABLE 1. Sample and culture results**

| Day | Action  | Sample | Bacteria                      | AMX | AMC | AN | ATM | AZM | CAZ | CRO | CIP | CLO | CFP | CFX | CZM | ETP | FEP | GEN | IMP | MEM | MIN | TET | CLM |
|-----|---------|--------|-------------------------------|-----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 0   | MLT starts | 8      | Leech conservation (foot)     | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| 8   |          |        | Acinetobacter johnsonii      | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| 8   |          |        | *Aeromonas* veronii          | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| 10  | Thumb (wound) | 8      | *Moraxella* osloensis       | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| 10  | Thumb (wound) | 8      | *Stenotrophomonas* maltophilia | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| 20  | Thumb (wound) | 8      | *Myroides* odoratus        | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| 26  | Thumb (wound) | 20     | *Stenotrophomonas* maltophilia | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| 35  | Thumb (wound) | 26     | *Aeromonas* salmonicida     | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | R   | S   | R   | S   | R   | S   | R   | S   | R   |

Numbers in parentheses refer to MIC, expressed in mg/L. CLSI, Clinical and Laboratory Standards Institute; MIC, minimum inhibitory concentration; MLT, medicinal leech therapy; SXT, sulfamethoxazole.

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together, likely explain the resistance to piperacillin/tazobactam. Using the plasmid-SPAdes assembler [24], we obtained a 21,555 bp contig containing the \(\text{bla}^{\text{SCO-1}}\), \(\text{bla}^{\text{CTX-M-3}}\), \(\text{bla}^{\text{TEM-1}}\) and \(\text{aac}(3)\)-II (conferring resistance to gentamicin) genes, together with genes associated with DNA mobility (Supplementary Fig. S1), supporting the location of \(\text{bla}^{\text{SCO-1}}\) on a mobile genetic element. We also found the aminoglycoside-modifying enzymes APH(3\(\beta\))-Ia (resistance to kanamycin) and Aad(A2) (resistance to streptomycin). Eventually the Sul and DfrA12 encoding genes (which confer resistance to cotrimoxazole, which the patient received for 3 days after the cessation of MLT), the Tet(E) encoding gene (resistance to tetracyclines) and the CatA2 encoding gene (resistance to chloramphenicol) were identified. The search for plasmid replicons using PlasmidFinder [10] returned negative for contigs obtained with both IDBA-UD and plasmid-SPAdes.

**Discussion**

* Aeromonas* spp. are natural colonizers of the digestive tract of leeches. Hence, since MLT has been used, the risk of infections due to *Aeromonas* sp. has been raised [11]. Accordingly, antibiotic prophylaxis, supplementation of the leech conservation medium with antibiotics or both has been proposed [12]. While infection with ciprofloxacin-resistant *Aeromonas hydrophila* during MLT has been reported [13], most *Aeromonas* strains appear to be susceptible to third-generation cephalosporins and piperacillin/tazobactam in this context [14]. In addition, several cases of infections due to ESBL (including CTX-M)-producing *Aeromonas* have been reported, but not in connection with MLT [15,16].

In our case, ASG1 was likely selected by the exposure to piperacillin/tazobactam and cotrimoxazole. The origin of ASG1 and of its acquired ARDs remains unknown because no other resistant strain was recovered from wound cultures. One possibility is that the plasmid could have been transferred from a transient bacterium from blood. Arguments against this hypothesis are that a rectal swab performed in the patient at day 38 was negative for any ESBL-producing *Enterobacteriaceae*, and the patient did not show any clinical sign of bacteraemia. Another possibility is that the water in which the leeches were bathed could have been contaminated either by ASG1 or another strain that could have transferred the SCO-1 plasmid.

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**FIG. 2.** Phylogenetic tree of 16S rRNA gene sequences encoding genes of *Aeromonas* species strain ASG1 and of *Tolumonas auensis* DSM_9187 (member of *Aeromonadaceae* family) to root tree. Tree was built using maximum likelihood (PhyML [4]) and iTOL online tool [5].
to ASG1. Nonetheless, other Aeromonas strains have been recovered from the leech conservation medium and from the wound, but neither ASG1 nor another A. salmonicida strain was identified. Possibly they may have been outnumbered by other bacteria. However, we are confident that the Aeromonas sp. strain isolated at day 20 and ASG1 was distinct, firstly because of their high MALDI-TOF MS scores (2.348 for Aeromonas sp., 2.154 for A. salmonicida strain ASG1), and secondly because of the different antibiotic resistance profiles, especially for fluoroquinolones (which is driven by the two chromosomal mutations found in the topoisomerases of ASG1, while the Aeromonas sp. strain was susceptible to fluoroquinolones).

Even more surprising is the presence of SCO-1, a carbencillinase that has rarely been reported [17–20]. The production of SCO-1 confers resistance to penicillins and to their combination with clavulenate but moderately decreases the susceptibility to piperacillin/tazobactam [18,19]. Of note, the genetic neighbourhood of blaSCO-1 was found to be similar to that of previous reports [17–20], being inconsistent with a new mobilization of blaSCO-1. The original host of SCO-1 remains unknown. Interestingly, as of this writing, SCO-1 is not included in the popular ResFinder [8] and CARD [21] databases, while it is in ARG-ANNOT [22] and MEGARes [23].

In conclusion, our observation suggests that antibiotic exposure can select for multidrug-resistant Aeromonas spp. during MLT, which warrants surveillance of the emergence of resistance during MLT.

Conflict of Interest

None declared.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.nmni.2017.10.005.

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