



Letter to the Editor

The glycerophospholipid-cholesterol acyltransferase gene (*gcat*) is present in other species of *Aeromonas* and is not specific to *Aeromonas hydrophila*



This letter concerns the article by Tsai et al. reporting a study in which the glycerophospholipid-cholesterol acyltransferase gene (*gcat*) was used in a cocktail of genes to target *Aeromonas hydrophila* using a multiplex PCR reaction (Tsai et al., 2019). The authors proposed that their new method is a rapid diagnostic tool for the detection of lethal pathogens like *A. hydrophila* in patients. However, the conclusions are not as straightforward as emphasized by the authors, because there is a high possibility of erroneous pathogen identification if the method in its current form is not revised. First, the newly identified strains in that study were not sequenced, curated, or submitted to a nucleotide database to enable comparisons and valid identification of *A. hydrophila*. Second, in the figures shown by the authors, the genes amplified in the multiplex reaction are not labelled for comparison with a labelled ladder.

A nucleotide search in the NCBI database with a 237-bp *gcat* gene sequence (GenBank accession number KM287428.1), similar in size to the fragment generated by the primers used by the authors, returned up to 54 hits. The reference sequence used scored a 100% match, as expected, and a number of strains with a match showed a percentage identity ranging from 86.92% to 98.73% for the sequences present in different *Aeromonas* species

(Table 1). Of note, even though some strains of *A. hydrophila* had a high identity match of 98.73%, other *Aeromonas* species, including *A. dhakensis*, *A. caviae*, *A. salmonicida*, and *A. encheleia*, had higher homology than one *A. hydrophila* strain 4AK4 (accession number CP006579.1), which had only an 86.92% match. It is not certain what *Aeromonas* species the authors would have identified if their isolates had been sequenced.

The nucleotide search conducted is in agreement with the original finding of Chacón et al., which showed that the *gcat* gene was present in all *Aeromonas* species (Chacón et al., 2002). Prior to that finding, the *gcat* gene was proposed by Buckley et al. as a toxin of *Aeromonas salmonicida* (Buckley et al., 1982). However, the gene was later found to be highly conserved among clinical and environmental isolates of *Aeromonas* species (Chacón et al., 2003). Many investigators have used the gene as a genus-specific and not species-specific marker in the identification of new isolates from diverse environments (Beaz-Hidalgo et al., 2010; Khor et al., 2015; Latif-Eugenín et al., 2016). Also phylogenetic analysis using 16S rRNA has shown that there is close clustering between *Aeromonas* species (Puthuchearry et al., 2012; Nwaiwu, 2018). This suggests that multiple methods should be used when *A. hydrophila*-specific identification for sensitive clinical application is desired until a realistic more specific method is found.

A revision by the authors to address the issues raised would be beneficial. If it is not possible to perform further work, it should be made clear that the multiplex PCR method described cannot distinguish between *Aeromonas* species.

Table 1Percentage identity for the 237-bp *gcat* gene sequence query (GenBank Accession number KM287428.1).

| <i>Aeromonas</i> species/ <i>gcat</i> gene sequence match | % identity | Accession number |
|--|------------|-------------------|
| <i>A. hydrophila</i> subsp. <i>hydrophila</i> ATCC 7966, complete genome | 98.73% | CP000462.1 |
| <i>A. hydrophila</i> gene for phospholipid-cholesterol acyltransferase | 94.94% | X07279.1 |
| <i>A. dhakensis</i> strain KN-Mc-6U21, complete genome | 97.47% | CP023141.1 |
| <i>A. caviae</i> strain R25-6 chromosome, complete genome | 96.62% | CP025705.1 |
| <i>A. caviae</i> GSH8M-1 DNA, complete genome | 95.36% | AP019195.1 |
| <i>A. salmonicida</i> strain O23A chromosome, complete genome | 92.83% | CP021654.1 |
| <i>A. salmonicida</i> strain S68, complete genome | 91.98% | CP022186.1 |
| <i>A. encheleia</i> strain NCTC12917 genome assembly, chromosome: 1 | 90.72% | LR134376.1 |
| <i>A. rivipollensis</i> strain KN-Mc-11N1 chromosome, complete genome | 86.92% | CP027856.1 |
| <i>A. hydrophila</i> 4AK4, complete genome | 86.92% | CP006579.1 |

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Ethical approval

Approval was not required.

Conflict of interest

No conflict of interest to declare.

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