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Letter to the Editor

Response to letter: Glycerophospholipid-cholesterol acyltransferase gene (gcat) sequence is present in other species of *Aeromonas* and not specific to only *Aeromonas hydrophila*

We thank to Dr. Ogueri Nwaiwu (2019) for his great interest and thoughtful review of our article. In his letter, he mentioned the multiplex PCR method that we used the glycerophospholipid-cholesterol acyltransferase gene (*gcat*) was not enable to make a specific and valid identification of *Aeromona hydrophila*.

Many virulence genes, such as heat-labile cytotonic enterotoxin (alt), serine protease (ser), heat-stable cytotonic enterotoxin (ast), and lipase (lip), were reported to have specifically associated with *A. hydrophila*; however, these genes also could be detected among *A. veronii, A. caviae,* and *A. salmonicida* (Khor et al., 2015; Onuk et al., 2013; Puthucheary et al., 2012). Although the gcat gene was found to be present in some Aeromonas strains and had a high identity match of *A. hydrophila, A. dhakensis, A.caviae, A. salmonicida,* and *A. encheleia* (Ogueri, 2019), some literature reported that the gcat gene was mainly found in *A. hydrophila* and *A. salmonicida* (Balakrishna et al., 2010; Onuk et al., 2013). In fact, monomicrobial necrotizing fasciitis (NF) caused by *A. hydrophila* revealed a high mortality rate of 50% in our institution (Tsai et al., 2012 and 2015), but we did find *A. salmonicida* in the human infections. These 5 primers were initially evaluated by the uniplex PCR for testing the tissue samples from the patients with positive wound culture by microbiological laboratory before multiplex PCR (Figure 1). Due to the high sensitivity and specificity of gcat gene in uniplex PCR, we selected the gcat gene to target *A. hydrophila* in multiplex PCR (Mendes-Marques et al., 2013).

Furthermore, Dr. Ogueri mentioned that the genes amplified in the multiplex reaction were not labelled with a labelled ladder in our figures. I add Figure 2 to clarify that we used 100 bp DNA ladder for labelling. This figure also explains the initial results of multiplex PCR by comparing the reference pathogen and tested sample from the NF patient.

Again, we would like to thank Dr. Ogueri for his comments on our study, and we can clarify the contents of this study. Indeed, to find a realistic, highly accurate and more specific method for quickly diagnosing lethal pathogens in NF patients is not easy. This study demonstrated that our multiplex PCR method for detection of these lethal microorganisms in tissue samples may be a useful diagnostic tool that revealed good results in microbiology laboratory. However, more research must be done to provide further results in clinical practice.

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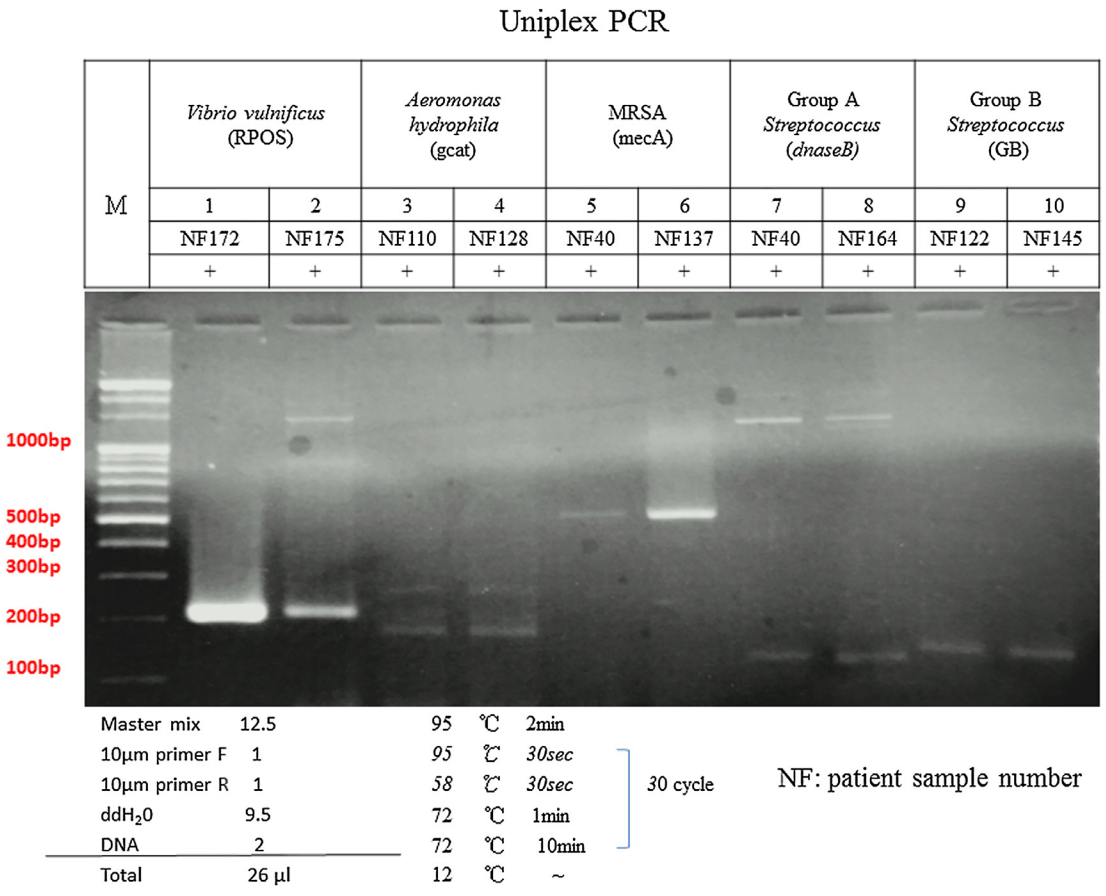

Figure 1. Uniplex PCR.

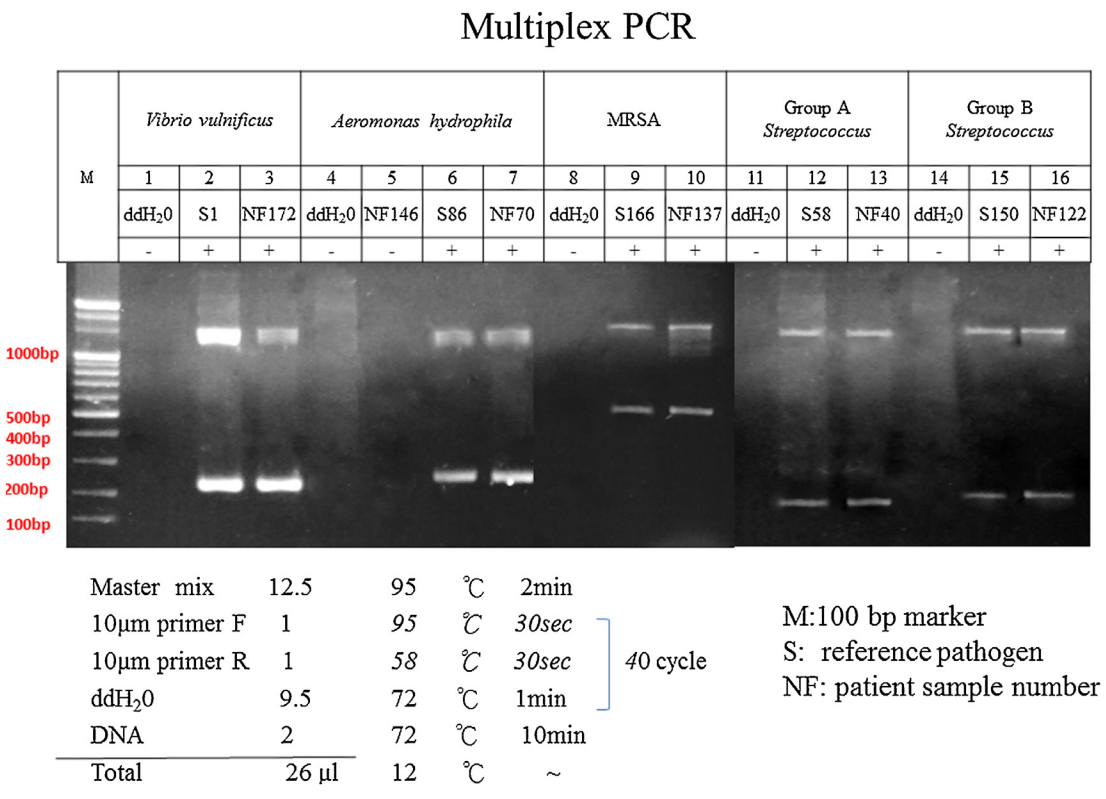

Figure 2. Multiplex PCR.