Supplementary Meterial
Molecular Detection, Quantification, and Toxigenicity Profiling of *Aeromonas spp*. in Source- and Drinking- Water

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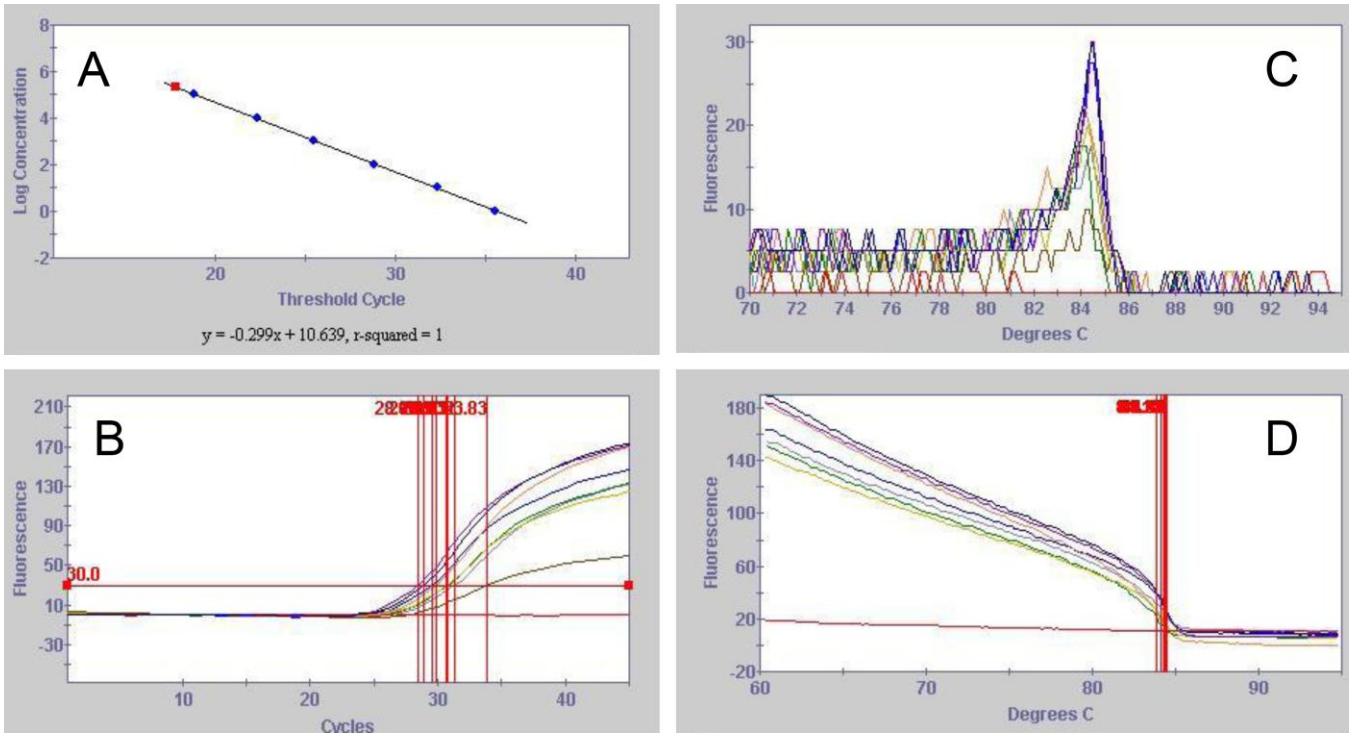

Fig. (S1). Genus-specific real-time quantitative PCR for detection and quantification of Aeromonads. Amplification was based on *Aeromonas* genus-specific primers targeting a 198 bp sequence in *gyrB* gene. **Panel A.** Standard curve prepared using Increasing amounts of DNA corresponding to varying number of cells (10^0-10^6) of *Aeromonas hydrophila* (type strain ATCC 7966). **Panels B-D.** Melting curve analysis on amplification products of *Aeromonas* samples.

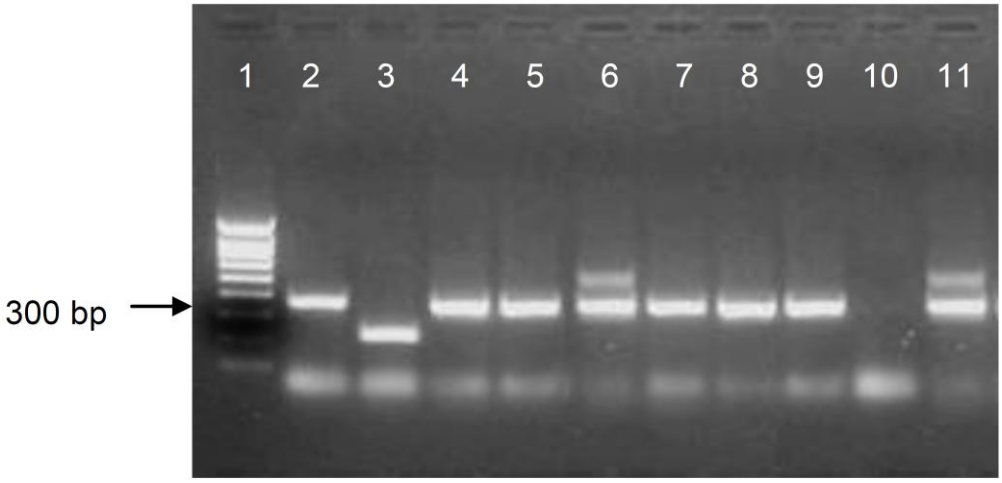

Fig. (S2). Multiplex PCR-based enterotoxin (*act*, *alt* and *ast*) profiling across *Aeromonas* species reference strains. Amplification was performed at 69ºC annealing temperature using the conditions described under Materials and Methods. Lane 1, 100-bp DNA ladder (Invitrogen); lane 2, *A. caviae* showing the *alt g*ene (361 bp amplicon); lane 3, *A. veronii* showing the *act* gene (232 bp amplicon); lanes 4-5, *A. media and A. jandaei* showing the *alt* gene (361 bp amplicon); lane 6, *A. hydrophila* showing the *alt* and *ast* genes (361 and 536 bp

amplicons, respectively); lanes 7-9, *A. sobria, A. eucrenophila, and A. trota* showing the *alt* gene (361bp amplicon), lane 10, negative control; and lane 11, positive control with *A. hydrophila* showing the *alt* and *ast* genes (361 and 536 bp amplicons, respectively).

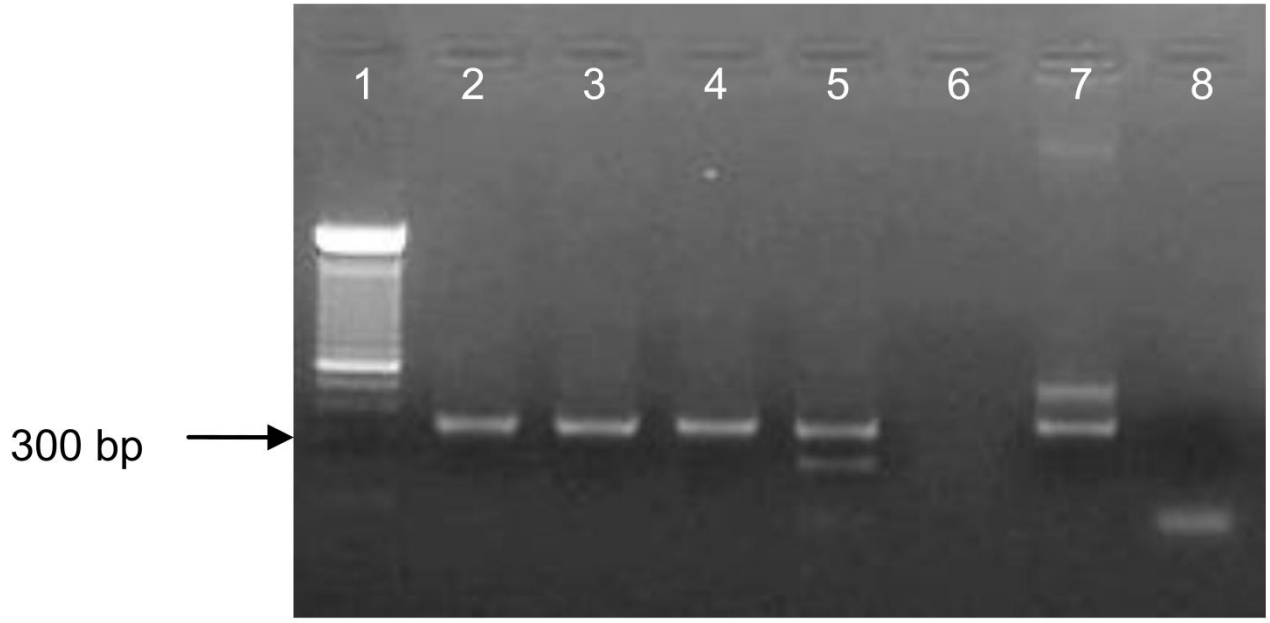

Fig. (S3). Multiplex PCR-based enterotoxin (*act, alt* and *ast*) profiling of *Aeromonas* isolates. Amplification was performed at 69ºC annealing temperature using the conditions described under Materials and Methods. Lane 1, 100-bp ladder (Invitrogen); lane 2-4, Isolates R'1B, R'4B and R'3A showing the *alt g*ene (361 bp amplicon); lane 5, Isolate DS'1A showing the *alt* and *act* genes (361 and 232 bp amplicons, respectively); lane 7, positive control with *A. hydrophila* showing the *alt* and *ast* genes (361 and 536 bp amplicons, respectively) and lane 8, negative control.

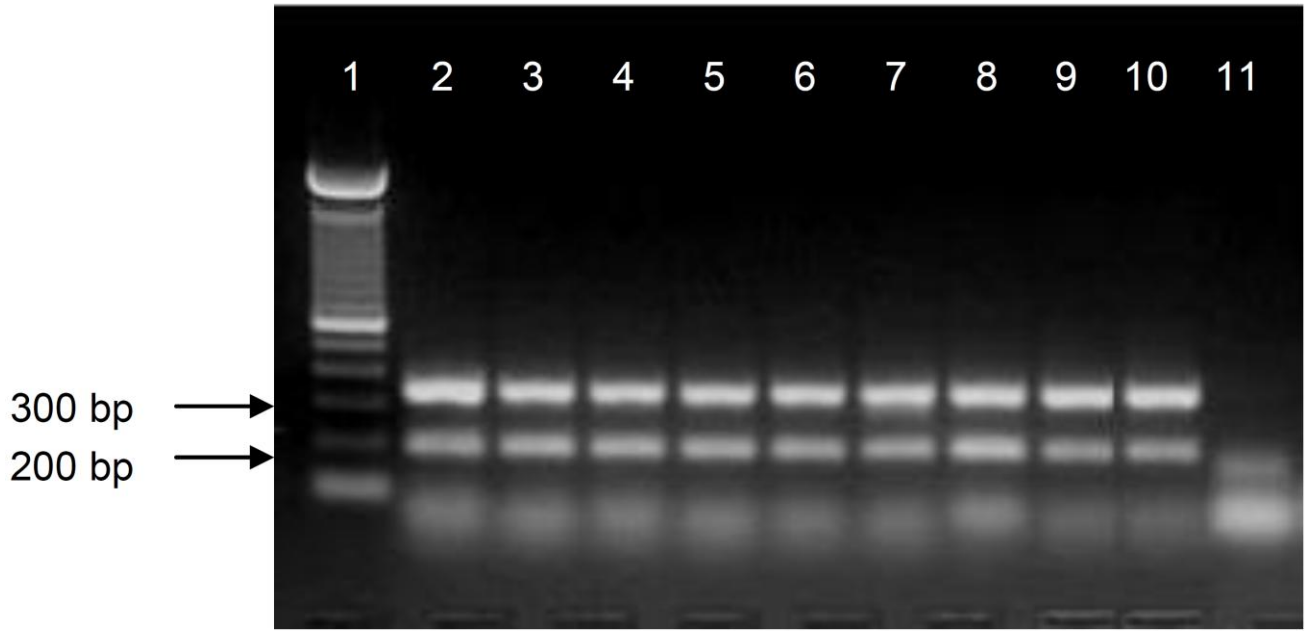

Fig. (S4). Multiplex PCR-based *16S rRNA* and *gyrB* gene pattern across *Aeromonas* spp. reference strains. Amplification was performed at 59ºC annealing temperature using the conditions described under Materials and Methods. Lane 1, 100-bp ladder (Invitrogen); lane 2-9, *A. caviae*, *A. veronii*, *A. media*, *A. jandaei*, *A. hydrophila*, *A. sobria*, *A. eucrenophila*, and *A. trota* showing the *16S rRNA* and *gyrB amplicons* (356 and 198 bp amplicons, respectively); lane 10, positive control with *A.hydrophila* showing the 16S rRNA and gyrB *g*enes; and lane 11, negative control.

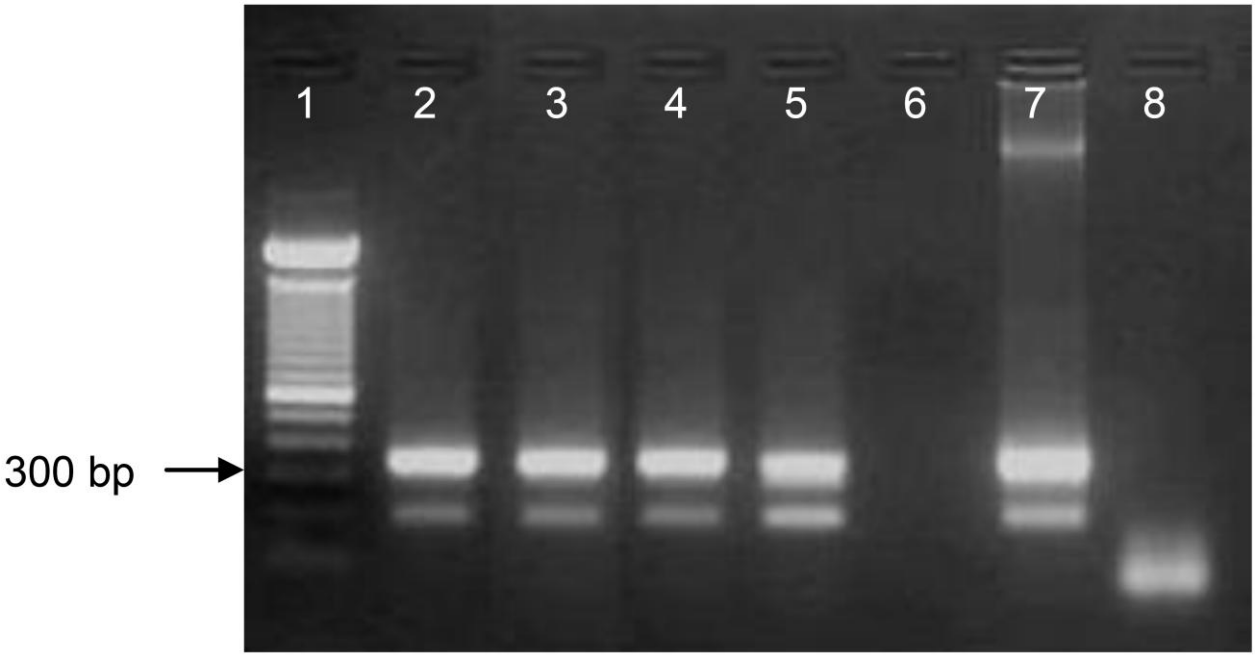

Fig. (S5). Multiplex PCR-based *16S rRNA* and *gyrB* gene pattern in water isolates of *Aeromonas*. Amplification was performed at 59ºC annealing temperature using the conditions described under Materials and Methods. Lane 1, 100-bp ladder (Invitrogen); lanes 2-5, Isolates R'1B, R'4B, R'3A and DS'1A showing the *16S rRNA* and *gyrB g*enes (356 and 198 bp amplicons, respectively); lane 7, positive control with *A. hydrophila* showing the *16S rRNA* and *gyrB g*enes and lane 8, negative control.

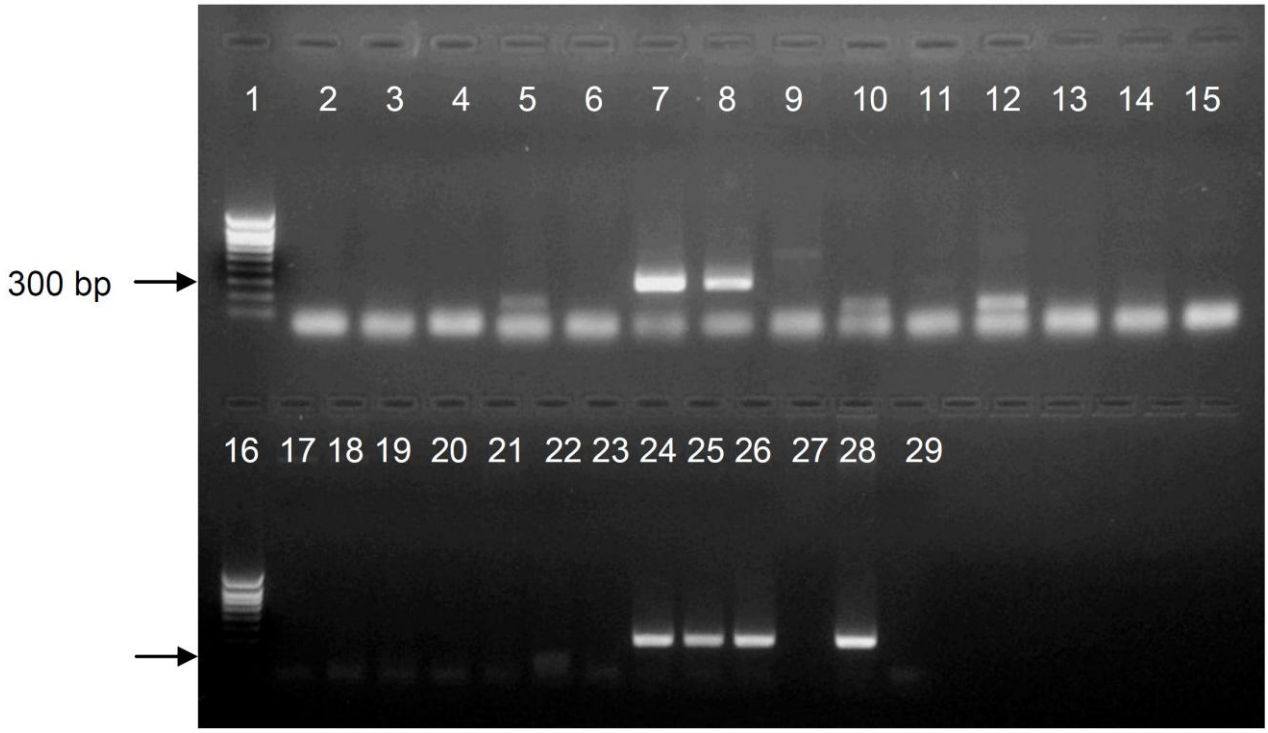

Fig. (S6). Singleplex PCR-based aerolysin (*aerA*) gene pattern across *Aeromonas* species reference strains and water isolates. Amplification was performed at 59ºC annealing temperature using the conditions described under Materials and Methods. Lane 1, 100-bp ladder (Invitrogen); lane 2-6, *A. caviae*, *A. veronii*, *A. media*, *A. jandaei*, *A.sobria*; lane 7-8, *A. hydrophila* and *A. eucrenophila* showing the *aerA* gene (309 bp amplicon); lanes 9-22, Water isolates ADA-VI-1, ADA-VI-2, ADA-VI-3, ADA-VI-4, ADA-VI-5, ADA-VI-6, ADA-VI-7, ADA-VI-9, ADA-VI-10, ADA-VI-11, and ADA-VI-12 that do not show the presence of aerolysin gene; lane 23, isolate R'1B, lanes 24-26, isolates R'4B, R'3A and DS'1A showing the *aerA* gene (309 bp amplicon), lane 28, positive control; lane 29, negative control.

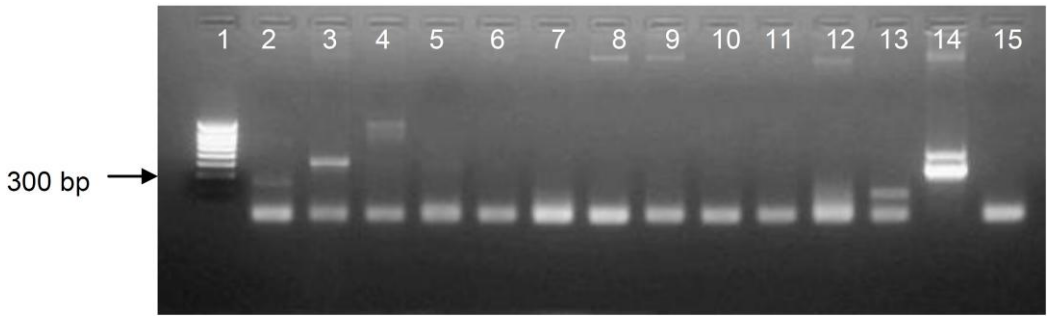

Fig. (S7). Multiplex PCR-based enterotoxins (*act, alt* and *ast*) profile in *Aeromonas* isolates. Amplification was performed at 59ºC annealing temperature using the conditions described under Materials and Methods. Lane 1, 100-bp ladder (Invitrogen); lane 2, Isolate ADA-VI-1 showing the *act* gene (232 bp amplicon); lanes 3 and 5, Isolates ADA-VI-2 and ADA-VI-4 showing the *alt* gene (361 bp amplicon); lanes 4, 6-13, Isolates ADA-VI-3, , ADA-VI-5, ADA-VI-6, ADA-VI-7, ADA-VI-8, ADA-VI-9, ADA-VI-10, ADA-VI-11, ADA-VI-12 showing lack of enterotoxin genes; lane 14, positive control with *A .hydrophila* showing the *alt* and *ast* genes (361 and 536 bp amplicons, respectively); lane 15, negative control.

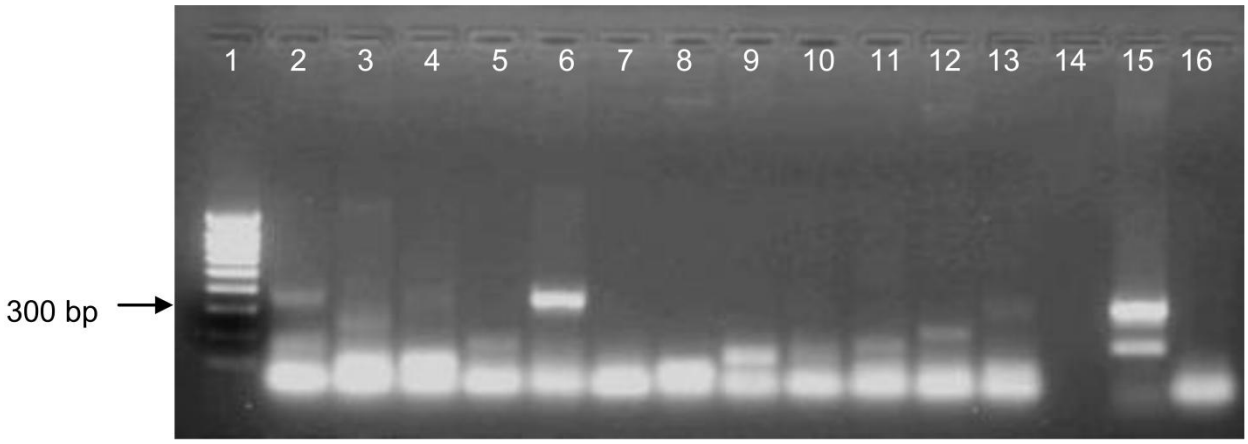

Fig. (S8). Multiplex PCR-based profiling of *16S rRNA, gyrB* and aerolysin (*aerA*) genes in *Aeromonas* isolates. Amplification was performed at 69ºC annealing temperature using the conditions described under Materials and Methods. Lane 1, 100-bp ladder (Invitrogen); lane 2, Isolate ADA-VI-1 showing the *16S rRNA* and *gyrB* gene (356 and 198 bp amplicons, respectively); lanes 5 and 11, Isolates ADA-VI-4 and ADA-VI-10 showing the *gyrB* gene (198 bp amplicon); lanes 6 and13, Isolates ADA-VI-5 and ADA-VI-12 showing the *16S rRNA* gene (356 bp amplicon); lanes 2-13, Isolates ADA-VI-1, ADA-VI-2, ADA-VI-3, ADA-VI-4, ADA-VI-5, ADA-VI-6, ADA-VI-7, ADA-VI-8, ADA-VI-9, ADA-VI-10, ADA-VI-11, ADA-VI-12 showing lack of aerolysin gene; lane 15, positive control with *A. .hydrophila* showing the *16S rRNA* and *gyrB* genes (356 and 198 bp amplicons, respectively); lane 16, negative control.