

ANTIBIOTIC RESISTANCE IN *Pseudomonas* spp. FROM KINNIYA AND WAHAWA HOT SPRINGS, SRI LANKA

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Pseudomonas are opportunistic pathogens that can cause human diseases. Hot springs are rich sources of thermophilic microorganisms adapted to natural geothermal environments. This study focused on identifying antibiotic-resistant genes (ARG) in selected *Pseudomonas* species isolated from the two hot springs, Kinniya (KY) and Wahawa (WH), Sri Lanka. Antibiotic sensitivity tests ($n = 17$) were conducted using the Kirby-Bauer disk diffusion method for aminoglycosides (kanamycin, gentamycin, and streptomycin) and quinolone ciprofloxacin. A real-time PCR technique was employed to detect resistance genes associated with aminoglycosides, phosphotransferase and acetyltransferase genes (*aph(3')-VI*, *aac(6')-I*, and *aac(3')-II*), as well as quinolone resistance genes (*gyrA*, and *parC*). Of the 17 strains (*Pseudomonas* sp., $n = 11$; *Pseudomonas aeruginosa*, $n = 6$), *Pseudomonas* sp. from KY displayed resistance to all antibiotics, while WH only resisted gentamycin and streptomycin. *Pseudomonas* sp. (75.54%) exhibited resistance to multiple drugs more frequently than *Pseudomonas aeruginosa* (25.33%). The most frequently identified ARG was *gyrA* (66.66%), particularly in KY isolates. Notably, *aac(3')-II* and *gyrA* were detected in 100% of WH isolates, whereas *gyrA* is the commonly found quinolone-resistance gene, and *aac(3')-II* is the aminoglycoside resistance gene. Findings reveal that antibiotic-resistant *Pseudomonas* isolates were less common in the WH than in the KY. The KY hot spring is more urbanized and populated by tourists than the WH. The KY isolates carry antibiotic-resistance genes, including all aminoglycoside-modifying enzymes and fluoroquinolone-resistant enzymes. However, WH isolates carry the aminoglycoside-modifying enzymes *aac(3')-II* and enzymes responsible for fluoroquinolone resistance: *gyrA* and *ParC*. The *gyrA* gene was commonly found among the selected two quinolone-resistance genes and *aac(3')-II* for aminoglycoside resistance. Monitoring anthropogenic activities regularly and thoroughly is critical to prevent the spreading of antibiotic resistance among environmental microbial communities.

Keywords: Aminoglycosides, Antibiotic resistance, Hot spring, *Pseudomonas* spp., Quinolone-resistance