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Occurrence of amikacin resistance in *Pseudomonas* sp. in selected hot springs in Sri Lanka

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Aminogly cosides, in combination with β -lactams, are commonly used as antipseudomonal agents due to their potential synergy with β-lactams. Amikacin is the most frequently used aminoglycoside for pseudomonal infections. This study aims to identify antibiotic-resistant genes associated with amikacin resistance in *Pseudomonas* sp. from hot springs in Sri Lanka. The geothermal hot springs: Kinniya (Trincomalee district) and Wahawa (Ampara district), were selected as study sites. The selected *Pseudomonas* sp. isolates: Kinniya (n = 15) and Wahawa (n = 2) were subculture in Muller-Hinton agar medium for an antibiotic-sensitivity test (ABST). The ABST was performed for kanamycin using the Kirby-Bauer disk diffusion method. The extracted DNA of isolates was then subjected to real-time PCR using amikacinresistant gene-specific known primers; aph (3')-VI and aac (6')-I, to identify the presence of these genes. Based on the ABST and PCR results, the Pseudomonas sp. isolates in Wahawa exhibited 100% susceptibility in both tests. The isolates from Kinniya exhibited resistance to kanamycin in 14 out of 15 cases (93.33%), with one isolate (6.33%) showing intermediate resistance in ABST. The aph (3')-VI resistant gene was found in 4 (26.66%) isolates, and aac (6')-I was found in 3 (20.0%) isolates. The aph (3)-VI gene was the most frequently detected gene (4 isolates) in Kinniya isolates. Ten isolates (10/15, 66.6%) harboured either of these two genes, and three isolates (3/15, 20.0%) harboured both. The ABST and PCR results were concordant among only ten isolates. Five isolates displayed resistance in ABST, however, did not exhibit the presence of the tested resistance genes in PCR results. The amikacin resistance in Pseudomonas sp. was 66.6% in Kinniya hot spring, while two Pseudomonas isolates from Wahawa were sensitive. The aph (3')-VI was the most observed amikacin resistance gene among the tested genes. The regulation of these genes and their related resistant pathways has to be studied further.

Keywords: Aminoglycosides, antibiotic resistance, hot spring, pseudomonas sp.